Ocellar and Extraocular Photoreceptors and Associated Interneurones in Insects

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Abstract

This thesis is based on the following four (I-IV) reports: I Ocellar fine structure in Caenis robusta (Ephemeroptera), Trichostegia minor, Agrypnia varia and Limnephilus flavicornis (Trichoptera). II Ocellar interneurones in the blowfly Calliphora erythrocephala: Morphology and central projections. III Interneurones subserving ocelli in two species of trichopterous insects: Morphology and central projections. IV Ultrastructure and central projections of extraocular photoreceptors in caddies-flies (Insecta: Trichoptera).

In report I the variation in fine structure of unsophisticated photoreceptors, the ocelli, is demonstrated. The variation is demonstrated firstly by differences in the optics and secondly by differences in the screening of the eye. In report II the morphology, pathways and terminations of the first-order ocellar interneurones are described in an insect species with the three ocelli situated closely together and having a common ocellar neuropile and nerve. Report III deals with the same subject but in two species with differing ocellar optics. The two species in turn differ from the one in report II by having well separated ocelli each with its own neuropile and ocellar nerve. The three species are compared and functional aspects discussed. In report IV the ultrastructure of the imaginal stemmata are studied. These are assumed to be the retained larval eyes in the imago. They are demonstrated to have crystalline bodies, rhabdoms and retinal axons projecting to their own neuropiles in the optic lobes. Presence of an S-antigen is demonstrated by immunocytochemistry. It is suggested that the imaginal stemmata might be still functioning photoreceptors, thus constituting a third visual pathway in addition to compound eyes and ocelli in caddies-flies.

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Signature Mariana Hagberg Date 1986-03-03

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This thesis is based on the following papers, which in the text will be referred to by their roman numerals.

I Ocellar fine structure in Caenis robusta (Ephemeroptera), Trichostegia minor , Agrypnia varia and Limnephilus flavicornis (Trichoptera). Eric Hallberg and Mariana Hagberg (1986) MS (page 24)

II Ocellar interneurones in the blowfly Calliphora erythrocephala : Morphology and central projections. Dick R. Nässel and Mariana Hagberg (1985) Cell Tissue Res 242:417-426 (page 43)

III Interneurones subserving ocelli in two species of trichopterous insects: Morphology and central projections. Mariana Hagberg and Dick R. Nässel (1986) Cell Tissue Res (in press) (page 53)

IV Ultrastructure and central projections of extraocular photoreceptors in caddiesflies (Insecta: Trichoptera). Mariana Hagberg (1986) Cell Tissue Res (in press) (page 79)

INTRODUCTION

The dorsal ocelli of insects have attracted the interest of scientists since 1741 when Reaumur made the first behavioral studies. Still the functional role of the dorsal ocelli is far from completly understood. Many adult insects possess dorsal ocelli in addition to the compound eyes. Generally there are three ocelli, one medially and two laterally situated, although one, two and four do appear. Insect orders exists which totally lack ocelli and also members within one and the same order may possess or be without ocelli (Kalmus 1945). Kalmus (1945) found a correlation between presence of wings and ocelli; if all species within an order possess wings they also possess ocelli and if all species within an order are wingless they are also anocellate. Also the development of the compound eyes - number of facets and size - is linked with the possession of wings.

An ocelli consists of a dioptric apparatus, photoreceptor cells, and a screening layer. The dioptric apparatus is a transparent cuticle which may form a lens, but also vitreous cells or a vitreous fluid may contribute to the dioptric apparatus. Crystalline bodies have not been found to be formed in ocelli. The shape of the lens varies and can be convexo-concave or biconvex. The lens is in all so far investigated species underfocused in respect to the retinal layer (Homann 1924, Wilson 1978), wich makes the ocelli unfit for image analyses. Instead the lens-system works as a condensor concentrating the light on the receptor layer. The photoreceptor cells are arranged in a shallow layer beneath the lens where they form rhabdoms. The ocellar capsule may be surrounded by pigment cells. Screening pigments can also be found within

the retinal cells.

The ocellar fields of wiew have been measured for Locusta migratoria and Calliphora erythrocephala (Cornwell 1955) and Austracis guttulosa (Wilson 1978). In C.erythrocephala the visual fields of wiew are in the female 206° laterally and 230° along the body axes. Males have slightly smaller fields of wiew due to their relatively larger compound eyes. The ocellar field of wiew is usually not as wide as that of the compound eyes.

The electroretinogram (ERG), with data on spectral sensitivity, has been measured for some species. In C.erythrocephala Kirchfeld and Lutz (1977) found a peak in 370nm (UV) and a considerable sensitivity up to 450nm (green). A marked UV peak is present in all so far investigated species and additional peaks are frequently found in the green region of the spectrum.

The axons of the photoreceptor cells converge onto the first order ocellar interneurones in the distal ocellar plexus. This is connected to the brain by means of the ocellar nerve, in which the ocellar interneurones run. These are cathegorised into large- medium- and small-diameter neurones based on axon diameter (Cajal 1918). The number of ocellar second order interneurones is small compared to the number of receptor cells, resulting in a high degree of convergence. In the desert locust, for instance, there are 600-800 retinula axons and 6-7 large-axons, 20 medium- and 70-80 small-diameter axons of which not all are secondary order neurones (Patterson and Goodman 1974). The ocellar interneurones project into the brain or proceed down the cervical connectives to terminate in the thoracic ganglia. For further description se papers II and III.

Several hypotheses have been put forward about the functional role of the ocelli. In contrast to earlier workers, Homann 1924, showed that the ocelli were incapable of form perception and suggested them to be registrators of brightness. A function as a stimulatory organ in the sense that an increase in light induces an increase in central exitability has often been suggested, however no conclusive evidence has been shown. Wilson 1978, and Simmons (1980) suggested a role for the ocelli in stabilizing flight by monitoring horizon movements during flight, based upon the contrast between the strong UV radiance of the sky and the strong UV absorbance of the ground. The visual component of equilibrium control of flying insects consists of inputs from a dual system; ocelli and compound eyes. The lack of spatial resolution in ocelli makes them better fit for detection of rapid changes of the visual field than the compound eyes with high spatial resolution (Laughlin 1974, Stange and Howard 1979). A role of moth ocelli in timing flight initiation at dusk by providing information of light intensity levels was suggested by Eaton et al (1983). Thus a fine tuning of the timing of flight initiation at the onset of nocturnal activities is possible. In the desert ant, Cataglyphis, the ocelli have been shown capable of detecting polarised light (Fent and Wehner, 1985) used for orientational purposes.

One cannot assume ocelli to have analogus functions in all species. The directions of ocellar fields of wiew and the pathways of ocellar interneurones differ among insects. Probably insect ocelli subserve more than one single function. The analysis of ocellar morphology, neuroanatomy and physiology should be placed in close context to the biology of the insect in order to elucidate ocellar function.

In some holometabolous orders the larval eyes are retained in the imago. They can remain as pigmented cells with or without rhabdoms within the optic lobes of the brain. In representatives of Trichoptera they are situated outside the brain close to the compound eyes. They are referred to as imaginal stemmata. Possibly they comprise a third visual pathway in addition to the compound eyes and the ocelli.

This thesis is concerned with the ocellar morphology and the projectional pattern of the first order interneurones in a) Calliphora erythrocephala (Diptera) and b) Agrypnia varia and Limnephilus flavicornis (Trichoptera). The morphology and central projections of the imaginal stemmata of Agrypnia varia was investigated as well.

MATERIALS AND METHODS

Calliphora erythrocephala (Diptera), Limnephilus flavicornis, Agrypnia varia, Trichostegia minor (Trichoptera) and Caenies robusta (Ephemeridae) have been studied in the different reports (I-IV). In this summary of the thesis only the three species C. erythrocephala, A. varia and L. flavicornis will be discussed. Ocellar morphology was studied using conventional standard light microscopical and electron microscopical methods. (See paper I under Materials and Methods for further specifications.) In order to study neuronal pathways and neuronal morphology, special neuroanatomical techniques were used. Horseradish peroxidase (HRP), a heme peptide, was used on C. erythrocephala. It can be used as a marker for light- and electronmicroscopy. HRP is taken up by intact or injured neurones and diffuses through the nerve cells, thus marking the entire cell (Nässel 1983). Transneuronal passage takes place

in vivo. In these cases HRP passes into certain sets of adjoining neurones wich are in synaptical contact (Nässel 1981). Primary and secondary marked neurones can be separated lightmicroscopically based on the relative intensity of the labelling. Ocellar interneurones were exposed to HRP by injuring the neurones at the site of the sense organ, the ocellus, and applying a drop of HRP solution at the site. (For further technical details se papper II.) Another neuroanatomical marker used was cobalt cloride. Cobalt ions can be taken up by intact and injured neurones and under certain conditions trans-synaptic staining can be obtained (Strausfeld and Obermayer 1976). Cobalt ions label also fine branches and terminations thus giving a very complete picture of the neurone. Ocellar neurones were exposed to cobalt cloride by the same procedure as for HRP. One drawback with both cobalt and HRP marking is that some neurones in a given tract may escape labelling. Hence, alone these methods cannot be used quantitatively even if the same number of neurones become reproducibly labelled in a large number of specimens. (For further details se papers II and III).

Immunocytochemistry was performed in order to trace the pathways of the receptor cell axons of the imaginal stemmata in representatives of Trichoptera. A rabbit antiserum, raised against bovine S-antigen, was used followed by the indirect peroxidase antiperoxidase technique (PAP) (Sternberger 1979; Korf et al. 1984) for detecting S-antigen-like immunoreactivity. The method gives an exellent marking of the receptor cells as well as their axons. Howewer the functional meaning of the presence of S-antigen is not yet fully understood. Although it indicates the presence of at least a part of the enzymatic apparatus of rhodopsin-based photoreception it cannot be considered as proof of a functioning photorceptor.

RESULTS AND DISCUSSION

Ultrastructure of ocelli in trichopterous insects. (Paper I).

In Agrypnia varia and Limnephilus flavicornis three dorsal ocelli are present. The dioptric apparatus differ in the two species. A. varia has a biconvex corneal lens and in L. flavicornis there is a convexo-concave corneal lens supplemented by an underlying haemocoelic space. The ocelli in both species are surrounded by longitudinally arranged tracheoles. In both of the species the rhabdoms are built up by four retinula cells, with orthogonally arranged microvilli. Lamellae extend between the retinular cells from cells with basaly situated nuclei.

The two different types of ocellar dioptrics were previously found within the order Trichoptera by Ehnbom (1948). He considered the type with a convexo-concave corneal lens and a liquid body as the most primitive of the two types. Functionally the design of lens system appears to be less critical since it in none of the cases produce an image on the receptor layer (D.-E. Nilsson personal communication). The tracheolar layer surrounding the ocelli probably screens the retinular cells by means of reflection. They may also have a tapetal function, i.e. light entering the ocellus but not being absorbed by the rhabdoms may be reflected back.

Morphology and projectional pattern of ocellar interneurones. (Papers II and III).

The blowfly, Calliphora erythrocephala.

In C. erythrocephala the retinula cells project to the distal ocellar neuropile which is common to all three ocelli and connedted to the brain by means of a single ocellar nerve. In the distal ocellar neuropile contacts are made with interneurones. These interneurones were found to consist of twelve large-diameter (8-10μm) neurones, ten medium-diameter (3-5μm) neurones and an uncertain number of small-diameter (less than 1,5μm) neurones.

The twelve large-diameter neurones all terminate in the posterior slope region of the brain, where three separate termination areas can be recognised: 1) the dorso-lateral, 2) the ventro-lateral and 3) the ventro-medial. Accordingly the twelve large-diameter neurones are classified into four types. When distinguishing ipsi-lateral from contra-lateral axons six types of large-diameter neurones are distinguished. (The termes ipsi- and contra-lateral are here used to indicate whether an axon crosses the midline of the brain or not.)

The ten medium-diameter neurones project to four different regions of the central nervous system. Two neurones terminate in each medulla, one neurone terminates dorsally in each lateral protocerebrum and one neurone ends basaly in each lobula, finaly one pair of neurones projects through the cervical connectives to the dorsal parts of the pro- and meso-thoracic ganglia.

Small-diameter neurones form three termination areas of their own: two bilateral symmetrical anterio-ventral to the posterior slope and one fused medial, dorsal to the oesophagael foramen. Small-diameter fibres also accompany medium-diameter fibres into the lobula and the medulla.

The ocellar interneurones were found to project to at least seven main regions of the CNS; 1) the posterior slope (three subregions), 2) the medulla, 3) the lobula, 4) the pro- and meso-thoracic ganglia, 5) the dorsal and lateral protocerebrum, 6) a region dorsal to the oesophageal foramen, 7) areas antero-ventral to the posterior slope.

The two subregions of the posterior slope where several large-diameter interneurones terminate, the ventro-lateral and the ventro-medial termination areas, coinside with dendritic arbors of sets of descending neurones connecting the brain to the thoracic ganglia (Strausfeld 1976, Strausfeld et al 1984). The dorso-lateral termination area coincides with the posterior optic tract which comprise inter alia contralateral and bilateral visual interneurones (Strausfeld 1976). This separation of the posterior slope into three termination areas suggests that outputs from large-diameter neurones may diverge onto at least three functionally separated pathways.

The medium-diameter neurones projecting to the medulla and lobula probably interact with optic lobe interneurones at their respective sites. Whether these connections are afferent or efferent is not yet known. The medulla-projecting neurones arborize only in the ventral third of the medulla, corresponding to the same portion of the compound eye. Thus there might exist an interaction between the dorsal visual fields subserved by the ocelli and the ventral part of the compound eyes. In the lobula the interaction with the ocellar interneurones may

involve the entire projected visual field. It should be mentioned that many ocellar interneurones have been found (in e.g. bees) which are multimodal or bimodal (Milde and Homberg 1984). Neurones like the medium diameter neurones may derive inputs from several brain centers and have outputs on other pathways.

An other area contacted by medium-diameter neurones is the pro- and meso-thoracic ganglia. The pro-thoracic processes may contact large motor-neurones which probably run to neck muscles, while the meso-thoracic processes lie in a region where sensory and interneurones of the halteres form terminations of dendrites (see also Strausfeld and Seyan 1985). Medium-diameter neurones also form extensive branches in many regions of the protocerebrum, the functional orginazation of these projection areas is however little known; the midbrain centers of the protocerebrum may represent multimodal control centers with outputs onto descending neurones and neurones of the autonomic nervous system (Strausfeld et al 1984).

The small-diameter neurones in the two bilateral-symmetrical areas anteroventral to the posterior slope may partly overlap with endings of mechanosensory fibres (Strausfeld 1976).In the fused medial area, dorsal to the oesophagael foramen small-diameter neurones may interact with fibres running in a posterior commisure. The remaining small-diameter neurones invade areas which are innervated by large- and medium-diameter neurones as well.

The caddies-flies, Agrypnia varia and Limnephilus flavicornis

In these two species the three ocelli are distinct and well-separated. Each ocellus has its own neuropile and its own ocellar nerve projecting

to the brain. This makes it possible to fill single ocelli and thus describe its individual central projection. The morphology and projections of the interneurones in the two species were found to be very similar in terms of number and projectional pattern. They are described below.

Four large-diameter (10um) neurones were found to project from one lateral ocellus to the posterior slope of the brain. Of these three project ipsilaterally and one contra-laterally. Another set of three large-diameter neurones project contralaterally down the cervical connective to the thoracic ganglia. Two of these neurones send extensive, partially overlapping, arborisations into the posterior slope. From the median ocellus two pairs of large-diameter neurones project down to the posterior slope. The axons of the first pair run without crossing the midline of the brain to the posterior slope. Here each axon terminates in one medially situated termination area specific for the median ocellar projection and one laterally coinciding with the lateral area of the lateral ocellar projection. The other pair projects bilaterally within the posterior slope to lateral termination areas. The total number of large-diameter ocellar neurones is eighteen of which twelve remain within the brain.

Ocellar interneurones of medium-diameter were found. These neurones do not project to specific areas within the brain, but serve as pathways between the three ocelli. Three such neurones were found connecting the median ocellus to the two lateral ones.

Three sets of small-diameter ocellar interneurones can be distinguished. One set projects from one lateral ocellus to the contra-lateral lobula and medulla of the optic lobes. Another type of small neurones was found

to project to the dorsolateral protocerebrum. Some small ipsilateral neurones invade a region ventral to the posterior slope. Also bundles of small-diameter neurones can be traced which connect the lateral and median ocellar neuropile.

As for the connections of the ocellar interneurones in the different projectional areas only suggestions and comparisons with other insects can be made since no other pathways are actually known in the brain of Trichoptera. The complexity in the morphology of the ocellar neuronal system of the two representatives of Trichoptera implies ocellar function similar to those of C.erythrocephala. In the flies, bees and locusts ocellar interneurones contact descending pathways with convergent multimodal inputs. Similar pathways are likely to exist also in caddiesflies. The complexity of the posterior slope termination areas and the projection of ocellar interneurones to many other termination areas imply that the ocelli feed input into many pathways. An integrative and relaying function is suggested for the ocellar interneurones.

Comparison between the blowfly and the caddies-flies.

The dioptrics of the ocelli differ between the two representatives of Trichoptera, the anatomy of the neuronal pathways, however, are assumed to be the same in the two species. The dioptrics of the blowfly ocelli are similar to that of one of the caddies-flies in having a biconvex corneal lens. The blowfly exhibit a major difference in gross morphology of the ocelli in that the three ocelli are situated close together and are served by a common distal neuropile connected to the brain by a single ocellar nerve in contrast to the two Trichoptera species where the three well separated ocelli each posses its own distal neuropile and

its own ocellar nerve. In all three species large-diameter ocellar interneurones project into the brain to terminate in subregions of the posterior slope. The median termination area is specific for the median ocellar termination in caddies-flies.

In the Trichoptera insects totaly six more large-diameter neurones, three from each lateral ocellus, have been traced. These are the descending neurones terminating in the thoracic ganglia. All three project contralaterally within the brain. In C.erythrocephala the contact between ocelli and thoracic ganglia is carried out by one pair of contralaterally projecting medium-diameter interneurones. Another difference in this contact between ocelli and thoracic ganglia is that in C.erythrocephala one single branching process, is sent into the posterior slope region from each axon while in the two Trichoptera insects large, extensive branches are sent into this region by four of the totally six neurones.

In C.erythrocephala another four pairs of medium-diameter neurones were found terminating in lateral protocerebrum and the optic lobes. No such medium diameter neurones were found in the two representatives of Trichoptera, but small-diameter neurones were found to terminate in the same areas. Here one and the same neuron contacts the medulla as well as the lobula. In C.erythrocephala this is done by separate neurones. The only medium-diameter neurones found in the two Trichoptera species interconnect the three ocelli. This type of neurones were not found in the blowfly at all. In the blowfly the contacts between ocelli may be carried out in the fused distal neuropile by processes of the large-diameter interneurones.

The termination area of small-diameter interneurones ventral to the

posterior slope in caddies-flies corresponds to the area anterio-ventral to the posterior slope in the blowfly.

Comparison with other insects.

The basic pattern of projections of the ocellar interneurones is the same in those insects studied to date: first order interneurones contact the following areas in the brain: The posterior slope, the optic lobes, the lateral protocerebrum and the ocellar neuropiles. In different insects this is done by constellations of neurones varying in number, size and arborisation pattern. In some insects ocellar interneurones project to additional areas, e.g., protocerebral bridge and antenno-glomerular tract (bees, locusts, crickets and cockroaches), ventral bridge (locusts, moths and bees), and tritocerebrum (locusts, moths, bees, blowflies and caddiesflies), in the vicinity of the posterior optic tract (cricket, cockroach and blowfly) (Eaton and Pappas 1978; Goodman 1976; Goodman and Williams 1976; Goodman et al 1975; Pan and Goodman 1977; Pappas and Eaton 1977; Goodman 1981; Koontz and Edwards 1984; Milde 1984, Nässel and Hagberg 1985). Furthermore, first-order ocellar interneurones proceeding to the thoracic ganglia have been reported in the bee (four pairs of contralaterally projecting neurones and one pair of ipsilaterally projecting neurones from each lateral ocellus and one pair of contralaterally projecting neurones from the median ocellus) (Pan and Goodman 1977) and in the blowfly (one pair of contralaterally projecting neurones (Nässel and Hagberg 1985)). Agrypnia varia and Linmephilus flavicornis each have three pairs of contralaterally descending large-diameter neurones. In the blowfly and in the bee these neurones are of medium size but in many insects they seem to be totally lacking. Within the brain of the hemimetabolous orders Blattoidea and Orthoptera all the ocellar interneurones project

ipsilaterally. In the holometaboulous orders Diptera, Hymenopthera, Trichoptera and Lepidoptera ocellar interneurones are found to project ipsi- as well as contra-laterally within the brain. The difference of morphology and projections between interneurones from median and lateral ocelli is pronounced in caddies-flies. No descending neurones or neurones to the optic lobes originate in the median ocellar neuropile and the ipsilaterlly projecting interneurones form an additional termination area not invaded by lateral ocellar interneurones.

Imaginal stemmata. (Paper IV).

In representatives of Trichoptera the larval eyes remain in the imago at different stages of reduction. Crystalline bodies and a monolayered rhabdom were found to exist in Agrypnia varia. S-antigen in the retinula cells and their axons was demonstrated by using immunocytochemistry whereby the central projections of the retinular cell axons also could be revealed. These were found to terminate either in the lamina accessoria or penetrate this to join the fibres of the outer chiasma of the optic lobes and terminate in the medulla accesoria. In C.erythrocephala no remnants of the larval eyes could be demonstrated using immunocytochemistry. Based on morphological and physiological grounds it is suggested that the imaginal stemmata in caddies-flies might be still functioning photoreceptors.

The imaginal stemmata might thus comprise a third visual pathway in addition to the compound eyes and the ocelli. A function in the entrainment of the circadian clock has been suggested (Schultz et al 1984). Howewer the function of this photoreceptor system is even more obscure than that of the ocelli.

CONCLUSIONS

The ocelli constitute a high-sensitivity non-spatial photoreceptor system, suited for detection of light intensities and rapid movments of the whole visual world. The first order ocellar interneurones of C.erythrocephala, A.varia and L.flavicornis terminate in areas where input onto descending neuron systems used in control of flight and head positioning is possible. They also terminate in various areas where multimodale interactions may take place. In conclusion the data shows that the ocellar pathways of C.erythrocephala, A.varia and L.flavicornis in general resembles those of previously studied insects. The detailed connections of these neurones to other neurones remain to be studied anatomically and electrophysilogically. Light perception in some insects appear to be subserved by three photoreceptor systems, compound eyes, ocelli and imaginal stemmata. The functional roles of the latter two systems need further studies for their elucidation.

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I

Ocellar Fine Structure in *Caenis robusta* (Ephemereoptera), *Trichostegia minor*, *Agrypnia varia* and *Limnephilus flavicornis* (Trichoptera).

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SUMMARY

Three dorsal ocelli are present in Caenis robusta (Ephemeroptera), Trichostegia minor, Agrypnia varia and Limnephilus flavicornis (Trichoptera). The dioptric apparatus of the ocelli differs among the four species. In Trichostegia and Agrypnia a biconvex corneal lens is present and in Limnephilus the corneal lens is convexo-concave complemented by an underlying haemocoelic space, whereas a cellular vitreous body is found between the cuticle and the retinal layer in the ephemerid. In the three trichopteroid species the ocelli are surrounded by an array of longitudinally arranged tracheoles; in Caenis there is a layer of screening pigments in this position. In this species the rhabdoms formed by microvilli of neighbouring retinula cells exhibit a meshwork pattern, obviously randomly arranged; in the three trichopteroid species the rhabdoms are isolated, being built up by four retinula cells. Cells with basally situated nuclei and lamellar extensions between the retinular cells are found in the ocelli of Trichostegia, Agrypnia and Limnephilus.

INTRODUCTION

Ocelli are present in many adult insects. These simple photoreceptors occur along with the structurally more sophisticated compound eyes, and are thought to function as the input channels for photic impulses governing certain behavioural mechanisms. The ocelli and their neuronal output have been shown to function as a steering agent for different behavioural events: spatial orientation during flight and walking, phototaxis, circadian activities etc. (Goodman 1981).

The photic perception of the ocelli is restricted to the perception of light/darkness; no image is formed. Usually the ocelli possess a lens system focused well below the retinal layer and which functions as a condenser lens, i.e. the retinal layer is illuminated evenly. A lens, formed by different light refracting structures in different species, and the light-sensitive structures, the rhabdoms, constitute all structural elements present in the ocelli. The rhabdoms are formed by the receptor cells. In addition there may be, in some cases, a tapetal layer consisting of reflecting pigment cells in the bottom of the ocellus. Even if the elements of the ocelli are few there is a considerable variation as regards the arrangement of these elements (Goodman 1981).

Recently there have been an increased interest in the ocelli regarding their physiology as well as the neuronal connections of the ocellar pathways. The ocellar interneurones can, due to the peripheral location of the ocelli, easily be stained with intracellular markers, and traced throughout the central nervous system.

This study of the fine structure of the ocelli of Caenis robusta, Trichostegia minor, Agrypnia varia, and Limnephilus flavicornis was made in conjunction with a study of the distribution patterns of the ocellar interneurones in the two species Agrypnia and Limnephilus. Also, this study should contribute to the rather sparse knowledge about the fine structure of the ocellar structures in adult insects.

MATERIALS AND METHODS

The three trichopterous species Trichostegia minor Curtis, 1834, Agrypnia varia Fabricius, 1793 and Limnephilus flavicornis Fabricius, 1787 and the ephemerid Caenis robusta Eaton, 1884 were collected at Stensoffa Ecological station 20 km east of Lund. Adult insects were caught during twilight using a mercury lamp.

The insects were decapitated and whole heads were fixed for light and electron microscopical studies. For light microscopy the specimens were fixed for 48 h in Bouins fluid, dehydrated in an alcohol series, embedded in paraffin, sectioned 10 μm and stained according to Delafield-Halmi (Romeis 1968).

For electron microscopy the preparations were fixed in 2.5% glutaraldehyde in 0.16 M sodium cacodylate buffer (pH 7.3) for 4 h at 4° C. The specimens were washed in the same buffer and postfixed in 1% osmium tetroxide in the same buffer for 2 h at 4° C. The tissue was dehydrated in alcohol and embedded in Epon. Ultrathin sections were cut with a diamond knife. The contrast of sections were enhanced by staining

with uranyl acetate and lead citrate in a LKB 2168 Ultrostainer. The sections were examined in a Zeiss EM 10 electron microscope.

RESULTS

In all the four species there are three ocelli present: one medial and two lateral. The medial ocellus is present between the antennal bases, and the two lateral ones are situated above, and medial to the compound eyes (Fig 2). The ocelli point in three directions with, in the horisontal plane, 90° between the longitudinal axes. The medial is thus directed forwards, the two lateral ones to the sides. Externally the ocelli can be discerned due to the presence of their corneal lenses which protrudes from the adjoining cuticle.

Caenis robusta

In Caenis the ocelli are surrounded by a pigment screen; a true corneal lens is lacking, the cuticle being underlain by a layer of vitreous cells which fill out the space between the cornea and the receptor layer (Fig 1A).

The corneal surface is convex; the thickness of the cornea is even throughout the entire surface area, about 2 µm. The cornea is underlain by an epidermal layer, which reaches to the receptor layer. The cytoplasm of the epidermal cells adjoining the cornea is electron dense, which abruptly is replaced by a very electron light cytoplasm (Fig 4). This part of the epidermal cells reach to the receptor layer and forms the so-called vitreous body. The lobed nuclei are situated in the

transition zone electron dark/light cytoplasm. The electron density of the distal cytoplasm is probably due to the presence of glycogen granules. Mitochondria and microtubules are also present in the distal part of the epidermal cells. The microtubules proceed in proximal direction, being distributed along the borders of the epidermal cells, often in bundles. Proximally the epidermal cells contain a finely granular substance in addition to the microtubules. The epidermal cells along the rim of the cornea do not possess any electron light proximal part; in this peripheral region the vitreous body is surrounded by an extracellular space in a collar-like fashion.

At the junction cornea/adjoining cuticle the epidermal cells contain electron dense pigment granules. This pigment layer continues both beneath the external cuticle and below it, around the ocellus. The pigment layer forms a continous sleeve around the receptor layer, except where the ocellar axons basally leaves the ocellus. The pigment shield measures 4-6 μm across and is peripheraly delimited by a thin basal lamina. At their inner borders the pigment cells of the shield penetrate as thin lamellae between the retinular cells (Fig 5). The pigment cells contain electron dense rounded granules (diameter about 1 μm). The electron density of the granules is variable from a very high even density to a moderate granular density. The pigment granules are densely packed within the pigment cells, between them there are restricted spaces of cytoplasm which is rather electron dense. Tracheoles are sparsely distributed within the pigment cells.

The retinular cells of the ocelli are present inside the pigment shield, below the proximal part of the vitreous body, which is formed by the epidermal cells. The contact zone epidermal cells/retinular cells is not marked with any specialized types of cell contacts such as desmosomes or

septate junctions. The lobed nuclei of the retinular cells are situated in the distal part of the cells, the rhabdoms are present below this level; further proximally the retinular cells pass through an extracellular space present in the basal part of the ocellus, before reaching the pigment shield. The distal part of the retinular cells houses, besides the pale nuclei a large number of mitochondria, and a fair number of small granules having the appearance of ribosomes and/or glycogen granules. The distance from the distal border of the retinular cells to the rhabdomeric level is 12-20 μm. The rhabdoms measure about 10 μm in length; in cross section the rhabdoms are rectangular, the length is usually 2-4 μm, whereas they measure about 2 μm across (Fig 3). The rhabdoms are developed where the retinular cells meet; at these borders the plasma membrane possesses microvilli (diameter about 65 nm). The interdigitating microvilli from adjoining retinulae cells together form the rhabdoms. The rhabdoms do not exhibit any regular pattern when seen in cross section, but is built up by an irregular meshwork of rhabdomeric lamellae. In the cytoplasm of the retinular cells there are low numbers of large, sphaerical granules (diameter 1-2 μm), which are moderately electron dense. These granules are not membrane-bound and probably represent lipid inclusions. The granules of this type are mainly found in the retinular cell cytoplasm below the rhabdom layer. Below the rhabdoms, there are extracellular spaces between the retinular cells; these spaces are partly filled with vesicular processes from retinular cells (Fig 6). As mentioned above the retinular cells penetrate the pigment shield in this basal part of the ocellus.

Trichostegia minor

In Trichostegia the ocelli are surrounded by an array of tracheoles, which are arranged along the long axis of the ocelli. The tracheoles

have diameter of 0.5-1 μm. The ocellus is distally delimited by a biconvex cuticular lens which has a considerably large thickness (Fig 1C). The cuticular lens is underlain by an epidermal layer, 2-4 μm thick. Apparently there is no direct contact epidermal cells retinular cells. However, the volume of the extracellular space here present may be influenced by processes during the fixation resulting in shrinkage of the tissues and consequently enlargement of extracellular spaces.

The retinular cells are peripherally surrounded by a thin cytoplasmic sheet which envelops the ocellus, the nuclei of these cells lie at the periphery of the ocelli. Distally and outside the cytoplasmic sheet there is a thin basal lamina; in the basal part of the ocellus the thickness of the basal lamina is considerable around the axons from the retinular cells. The nucleus of the retinular cells are lobated and situated in the distal part of the retinular cells. Mitochondria constitutes conspicous cellular elements at this distal level as well as around the rhabdoms. The rhabdoms are usually formed by four retinular cells, though diverging numbers of retinular cells (two or three) exist. The rhabdoms have in transverse section either square or rounded outline (Fig 7). In cross section the microvilli are arranged in such a way that microvilli from two opposed retinular cells are parallel meeting the microvilli from two other retinular cells perpendicularly along their rhabdomeric borders. The retinular cells are joined with desmosomes along the rhabdom edges. The spaces between the retinular cells of neighbouring units, ranging in thickness from 0.1 μm to 0.5 μm, are filled with the lamellar extensions from the cells mentioned above, which envelope the ocelli peripherally. The rhabdoms, about 20 μm long, extend to the proximal part of the retinular cells, which at this level turns into retinular axons.

Limnephilus flavicornis.

In Limnephilus the ocelli are situated in cuticular pockets protruding from the cuticle; the dorsal and ventral parts of the ocellus lying close to the cuticular elements. The corneal lens is less well developed compared to Trichostegia, not beeing biconvex but convexo-concave. Proximally to the cuticular lens there is a haemocoelic space surrounded by a single layer flattened cells. These cells peripherally surrounds the ocellus (Fig 1B). In none of our preparations the haemocoelic space fills up the room between the cornea and the retinula cells. The same considerations about the possible shrinkage apply also in this case. The distance corneal lens - receptor layer is larger in this species than in Trichostegia. However, a less developed corneal lens could be matched with a larger distance to the receptor layer. The diameters of corneal lens and ocelli are also smaller in Limnephilus than in Trichostegia in spite of larger total body length. As regards the arrangements of the retinular cells, rhabdoms, surrounding glial cells and basal lamina these are similar to the corresponding structures of the ocelli of Trichostegia. The tracheal sleeve around the ocellus is however more developed in Limnephilus than in Trichostegia (Figs 8,9).

Agrypnia varia

The general arrangement of the ocelli in Agrypnia conforms to that of Trichostegia (Fig 1C). Also the cuticular lenses have the same shape as in the latter species. The rhabdoms are built up by four retinular cells, and have in transverse section a cross-shaped outline (Fig 10). The rhabdomeric microvilli are orthogonally arranged as in Trichostegia and Limnephilus. Desmosomes join the retinular cells close to the rhabdom. Outside the cellular layer which surrounds the ocelli and the

adhering basal lamina there is an extremely well-developed array of tracheoles. The tracheoles are arranged parallel to the longitudinal axis of the ocellus.

DISCUSSION

In morphologically simple photoreceptors such as the ocelli of insects, the span of variation of the different elements is limited; there are few units that can be modified. Essentially there is in the ocelli the following structural/functional entities: light refractive structures (lens), light receptive structures (retinular cells, rhabdoms), and screening elements (absorbing pigments). The possible variation is thus confined to these three structural elements. The primary functional demand of the ocelli is reception of light; no image forming qualities are required, adding to the picture of an unsophisticated receptor system.

The lenses in the four studied species exhibit three different types of development. Trichostegia and Agrypnia have a biconvex cuticular lens, whereas Limnephilus has a convexo-concave cuticular lens complemented by a haemocoelic space. These two ocellar types represent the two patterns found within the order Trichoptera as classified by Ehnbom (1948) where the type with a convexo-concave corneal lens and a liquid body is considered the most primitive one of the two types. The third ocellar type is found in Caenis where the lens is formed by the epidermal layer, which probably contains glycogen granules, lending a light refractive capacity to these cells. This latter type of lens system has earlier been described in other ephemerid species as well as in other insects

(Link 1909). Functionally the lens system appears to be less critical, since it apparently just creates an even illumination of the receptor layer. The rhabdoms in insect ocelli are usually formed by the union of two or more rhabdomeres originating from adjacent retinular cells. In Manduca sexta and other sphinx moths (Dickens and Eaton 1974) the arrangement of the rhabdoms is aberrant; here the rhabdomeric microvilli projects into a central cavity of the single retinular cell. The rhabdoms of the ocelli can be arranged either randomly, as in Caenis or forming discrete units as in Trichostegia, Agrypnia and Limnephilus. Rhabomeric meshworks similar to that of Caenis is also found in Boettcherisca (Toh et al 1971), Rhodnius (Goodman 1981) and in Trichoplusia (Dow and Eaton 1976). Discrete rhabdomeric units formed by retinular cells have also been found in Periplaneta (Cooter 1975 ; Weber and Renner 1976; Toh et al 1983) in dragonflies (Ruck and Edwards 1964) and in the honeybee (Toh and Kuwabara 1974).

Screening pigments is usually present around the ocelli, in some cases the retinula cells themselves may contain screening pigments (Goodman 1981). In some species a tapetal layer of reflecting pigment is present in the bottom of the ocellar cup (Goodman 1981). In Caenis the ocelli are surrounded by screening pigment cells, whereas the ocelli in Trichostegia, Agrypnia and Limnephilus are surrounded by an arrangement of tracheoles; this tracheolar layer probably screens the retinular cells by means of reflection rather than by absorption, as is the case with the screening pigment in Caenis. Also the tracheols may have a tapetal function, i.e. light entering the ocellus but not being absorbed by the rhabdoms, may be reflected back. Systems of tracheoles are also found in the bottom of the compound eyes of lepidopterans (Ribi 1979), functioning as a quarter wave-length reflector. It has been shown that the screening pigment shield of the ocelli of dragonflies has an iris

function, migrating centrally at the distal part (aperture) of the ocellus (Stavenga et al 1979). In Caenis such movements are probably not present due to the presence of a vitreous body, whereas in the trichopterans the tracheal bundles ought to be stationary.

To summarize, this study has further demonstrated the variation in fine structure of this unsophisticated photoreceptor. Within given functional and structural limits, the design of this type of eye can be solved in different ways. One example is the dioptric apparatus designed to spread the incoming light evenly over the receptor layer. In Trichostegia and Agrypnia this is achieved by a thick biconvex cuticular lens, in Limnephilus by a convexo-concave cuticular lens complemented by a liquid filled space and in Caenis a vitreous body, formed by epidermal cells, serves the same purpose. An other example of this is the screen that is isolating the eye: in Caenis (and in many other insects) this is acheived with the pigment layer, whereas in the trichopteroid species a tracheolar system serves this function. Possibly also this screening function is supplemented with a tapetal function, reflecting the light within the ocelli.

ACKNOWLEDGEMENTS

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FIGURELEGENDS

Fig 1. Semischematic presentations of the ocelli of the investigated species. Scale bar: 50µm.

A. In Caenis the corneal cuticle (C) and the retinal layer (R) are separated by a vitreous body (VB), which is surrounded by an extracellular space (ES). The ocellar nerve (ON) passes through the pigment shield (shown stippeled). RH=rhabdoms.

B. In Limnephilus the corneal lens (C) is moderately developed. The retina (R) is resting on a cuticular shelf. ON=ocellar nerve, HS=haemocoelic space, RH=rhabdoms.

C. The ocelli of Trichostegia and Agrypnia have a similar structure with well-developed corneal lenses (C). The retinal layer (R) is present immediately below the corneal lens. ON=ocellar nerve, RH=rhabdoms.

Fig 2. Frontal view of the head of Limnephilus showing the corneal lenses of the ocelli (arrows). The arrangement of the ocelli is similar in the investigated species: there are two lateral ocelli above the compound eyes (CE), and one medial between the basal parts of the antennae (A). Scale bar: 200 µm.

Fig 3. Cross section through the rhabdom layer of the medial ocellus of

Caenis. The rhabdoms (RH) are formed by microvilli from two retinular cells (RC). The rhabdoms do not exhibit any regular arrangements. Extracellular spaces (ES) are present between the retinular cells. Scale bar: 1μm.

Fig 4. The cornea (C) in Caenis is underlain by hypodermal cells, which reach to the retinular layer. The distal parts (DP) of the hypodermal cells are more electron dense and contain nuclei (N), whereas the proximal part (PP) are less electron dense and form the so-called vitreous body. Scale bar: 2 μm.

Fig 5. In Caenis the ocelli are surrounded by a pigment shield, which contain pigment granules (PG) of varying shape and electron density. Thin processes from the pigment cells (arrows) are present betweeen the retinular cells (RC). Scale bar: 1 μm.

Fig 6. Longitudinal section through the rhabdoms (RH) of Caenis. Below the rhabdoms there are large extracellular spaces (ES), in which vesicular portions of retinular cells are present. Scale bar: 2 μm.

Fig 7. In Trichostegia the rhabdoms (RH) have a square outline in cross section, being formed by four retinular cells (RC). Glial cell processes (arrows) are present between the retinular cells. Scale bar: 1 μm.

Fig 8. The rhabdoms (RH) in Limnephilus are similar to those of Trichostegia. Peripherally the ocellus is enveloped in a thin layer of glial cells (GC), which have processes between the retinular cells (arrows). A prominent array of tracheoles (TR) are present around the ocellus. Scale bar: 1 μm.

Fig 9. Longitudinal section through the lateral ocellus of Limnephilus. In the bottom of the ocellus the well-developed basal lamina (arrows) is penetrated by the axons (AX) from the retinular cells. TR=tracheoles, RH=rhabdoms. Scale bar: 4 μm.

Fig 10. Transverse section through the lateral ocellus of Agrypnia. The rhabdoms (RH) are cross-shaped in this species. There are tracheoles (TR) outside the ocellus. Scale bar: 1 μm.

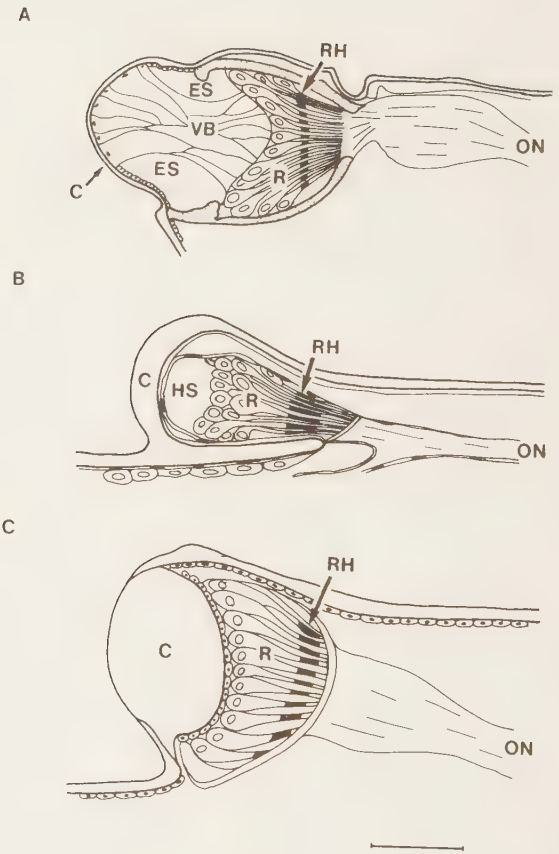

Fig 1

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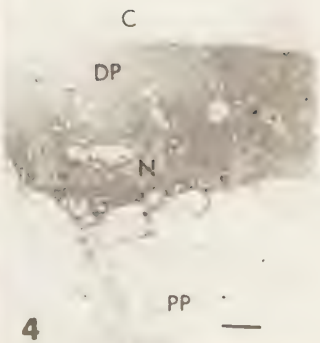
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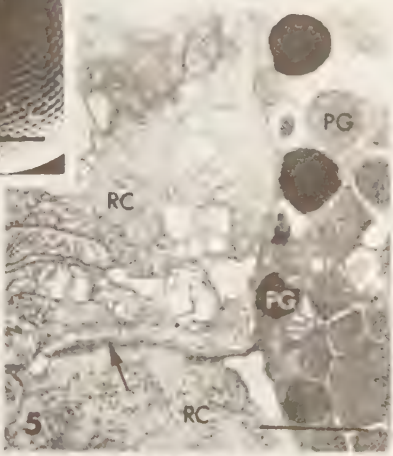
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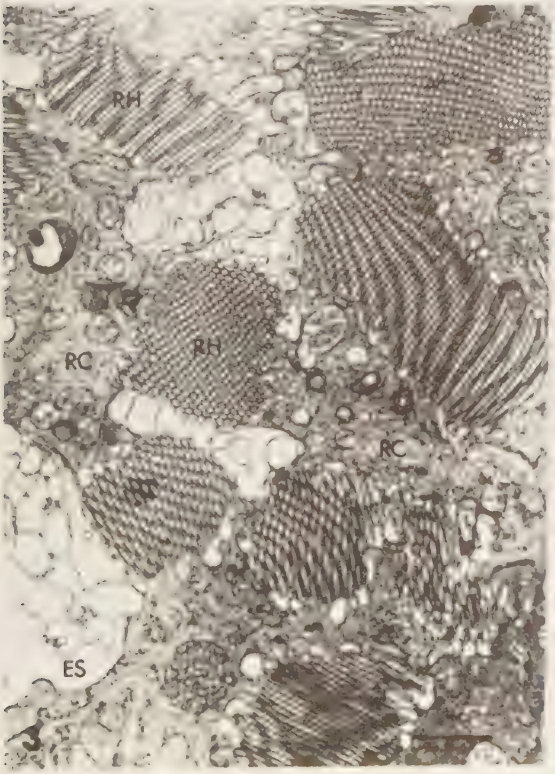
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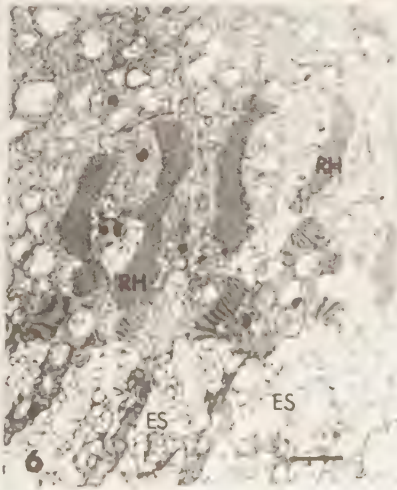
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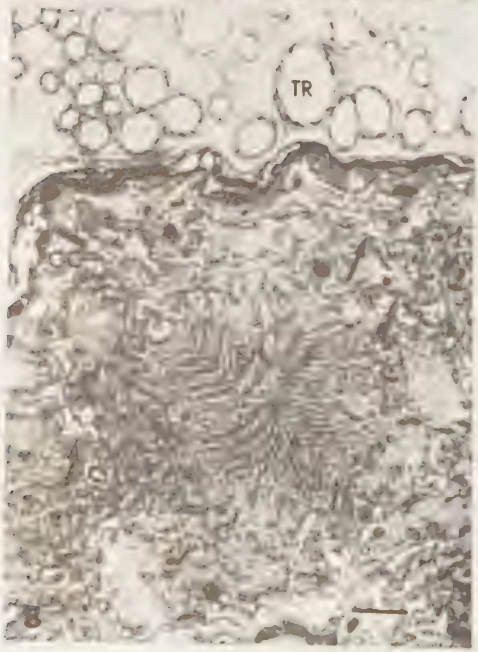
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II

Ocellar interneurones in the blowfly *Calliphora erythrocephala*: Morphology and central projections

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Summary. The morphology and central projections of first-order ocellar interneurones were analysed in the blowfly, *Calliphora erythrocephala* after cobalt and horseradish-peroxidase labelling. Three classes of interneurones can be distinguished on the basis of axon diameters: large, medium and small neurones. In total there are 12 large, 10 medium and an unknown number of small interneurones. These interneurones connect the fused first-order ocellar neuropil (underlying the three ocelli) with various areas of the central nervous system. The large neurones terminate in three subregions of the posterior slope (ocellar foci); the medium neurones arborise in several regions of the lateral protocerebrum, in the posterior slope, the lobula, the ventral medulla, and in the pro- and mesothoracic ganglia. The thin fibers arborise in all the above regions (except in the thoracic ganglia), and in addition in the neuropil dorsal to the oesophagus and antero-ventral to posterior slope (tritocerebrum). The anatomy of the ocellar pathway in *C. erythrocephala* is compared with those in other studied insects. Possible interactions between ocellar interneurones and other pathways are discussed.

Key words: Insect visual system - Ocellar pathway – Visual interneurones – Neural tracing – *Calliphora erythrocephala*

In addition to lateral compound eyes most adult insects possess dorsal ocelli. These simple photoreceptor organs have been ascribed many photoreceptive roles. Most authors agree that ocelli serve more than one function in an insect and that ocelli may have varying roles in different insect species (reviewed by Goodman 1981). At one extreme ocelli are totally lacking in some insects, many of which also lack wings (Kalmus 1945).

Behavioural and physiological experiments have indicated that ocelli mediate certain orientation behaviour, including compensatory head rolling (Wilson 1978a; Taylor 1981a, b; Rowell and Pearson 1983). Similar behaviour can, however, also be controlled by the compound eyes (Taylor 1981) and to an extent by mechanosensory inputs (Rowell and Pearson 1983). It has also been shown that ocelli interact with compound eyes to control posture and locomotion (Goodman 1970, 1981). Another function, proposed from experiments, is the registration of absolute brightness level or rate of change of brightness (Hoyle 1955; Schricker 1965). This information might be used to modulate neuronal activity in the optic lobes of the brain (Mimura et al. 1969) and for control of neurohormonal systems (Heinzeller 1976). Ocellar inputs have been shown by electrophysiology to converge with neurones of other systems: mechanosensory and olfactory (Horridge 1964; Mimura et al. 1970; Suzuki et al. 1976; Kondo 1978; Simmons 1980) and the optic lobes (Horridge 1964; Patterson and Goodman 1980; Simmons 1981).

How do the above-proposed functions of the ocelli correlate with what we know about the anatomy of the ocellar pathway? From a rather large number of anatomical studies (summarized by Goodman 1981; Milde 1984; Milde and Homberg 1984; Koontz an Edwards 1984) it emerges that the ocellar pathway is quite complex. The relatively simple first-order ocellar neuropil, with the photoreceptor synapses, is connected by means of afferent and efferent interneurones to a large number of regions of the CNS. Some of these projections could well be substrates for control of orientation behaviour: connections to descending neurones in the posterior slope of the brain and directly to thoracic ganglia (Pan and Goodman 1977; Goodman 1981; Koontz and Edwards 1984). Also connections between ocelli and optic lobes have been resolved that probably account for the above-described interactions between these regions (Goodman and Williams 1976; Goodman 1981; Koontz and Edwards 1984).

In dipterous insects large ocellar interneurones have been described mainly from Golgi impregnations in *Musca domestica* (Strausfeld 1976); only little information is available on the smaller neurones. Since flies have been used extensively in studies of visual orientation behaviour (see review by Hausen 1983) and other pathways in the brain have been quite well described anatomically (Strausfeld et al. 1984), we feel that more detailed analysis of the ocellar pathway is needed.

Materials and methods

Blowflies, *Calliphora erythrocephala*, were obtained from a laboratory culture. About 200 flies of both sexes were used for cobalt and horseradish-peroxidase (HRP) labelling of ocellar interneurones. The labelling with cobalt and HRP was performed as follows: The flies were mounted with dental wax onto a slide (dorsal side up). The ocellar lenses were cut off with a razor blade or pierced with a fine needle

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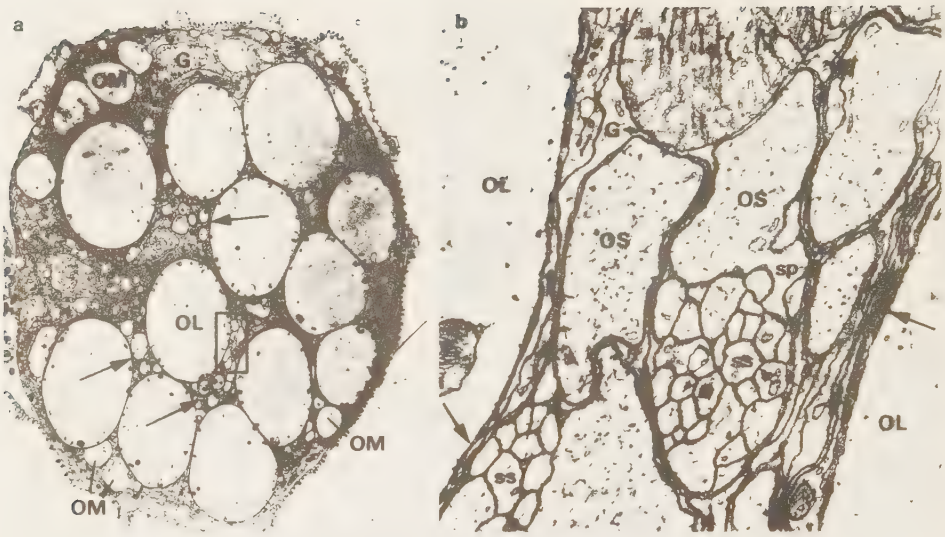

Fig. 1 a, b. Electron micrographs of cross sections of the ocellar stalk approximately half-way between the ocellar neuropil and the brain. **a** The optic stalk contains 12 large-diameter axons (*OL*), 10 medium-diameter axons (*OM*) and a number of small-diameter axons (*arrows*). Note in framed area some small fibres and two bundles of very fine fibres. ×1760 **b** Enlargement of the framed area in (**a**). Portions of two large- (*OL*) and some small- (*OS*) diameter fibres surrounding two bundles of very thin fibres (*ss*). These might arise as spines of branches (*sp*) from small fibres. Glial cells (*G*) form several layers around the OL-fibres (*arrows*). ×37600

deep enough to sever the peripheral ocellar neuropil. A small drop of 5% cobalt chloride in distilled water or 2% HRP in 0.1 M KCl was placed on the lesion and covered with vaseline. Three types of lesions were made: 1) one lateral ocellus, 2) the median ocellus, or 3) all three ocelli. Uptake time was 0.5 h for cobalt and 24 h for HRP, both at 4°C. Processing and silver intensification of cobalt-filled tissue was according to Bacon and Altman (1977). The HRP-labelled tissue was processed according to Nässel (1982, 1983). The cobalt-labelled tissue was either prepared for cleared whole mounts or, as the HRP-labelled tissue, embedded in Araldite and cut at 25 µm. Some brains, with attached ocellar stalk, were fixed for electron microscopy in 2.5% glutaraldehyde in 0.16 M sodium cacodylate buffer and 6% sucrose. These were postfixed in 2% osmium tetroxide and embedded in Epon; transverse sections of the ocellar stalk were cut with a diamond knife. Tracings of cobalt- and HRP-labelled neurones were made with a drawing tube fitted to a Leitz Orthoplan microscope.

Results

In *Calliphora erythrocephala* the three ocelli are arranged in a triangle on the vertex of the head between the compound eyes: one medial and two lateral ocelli. The axons of the ca. 300 photoreceptor cells of the three ocelli project to a common neuropil, the distal ocellar neuropil, situated immediately beneath the receptor layer (see Toh and Kuwabara 1974). This neuropil is a more or less complete fusion of the original three neuropils. The distal ocellar neuropil is connected to the brain by means of a single ocellar nerve or ocellar stalk. The neurones of the ocellar nerve continue inside the brain as a bilaterally symmetric tract. This tract divides into two bundles of axons deeper in the brain. The axons of the ocellar interneurones run between the calyces of the mushroom bodies and posterior to the protocerebral bridge. From this point axons run in different tracts to different regions of the brain and the thoracic ganglia.

In the electron microscope the ocellar nerve was found to contain 12 large-diameter (8–10 µm), 10 medium-diameter (3–5 µm), and an uncertain number of small-diameter (less than 1.5 µm) axons (Fig. 1a). In the nerve all axons are surrounded by glial elements. The large-diameter axons are often enwrapped in several layers of glial membrane (Fig. 1b). The 10 medium-diameter axons are arranged peripherally around the large-diameter axons. The small-diameter axons are distributed in a few groups centrally in the nerve among the large-diameter axons. Some profiles in the nerve have very small diameters (Fig. 1b). Many of these small diameter profiles are branches or spines from small-diameter axons (see Fig. 1b), and hence it is difficult to estimate the number of small-diameter axons.

Morphology and central projections of ocellar interneurons

Since the neuropil of three ocelli in *Calliphora* is fused, and filling of interneurones depended on damaging their dendrites or terminals, we could not reliably fill interneurones of single ocelli. Our data from lesions of single ocelli can thus only give a rough indication concerning which

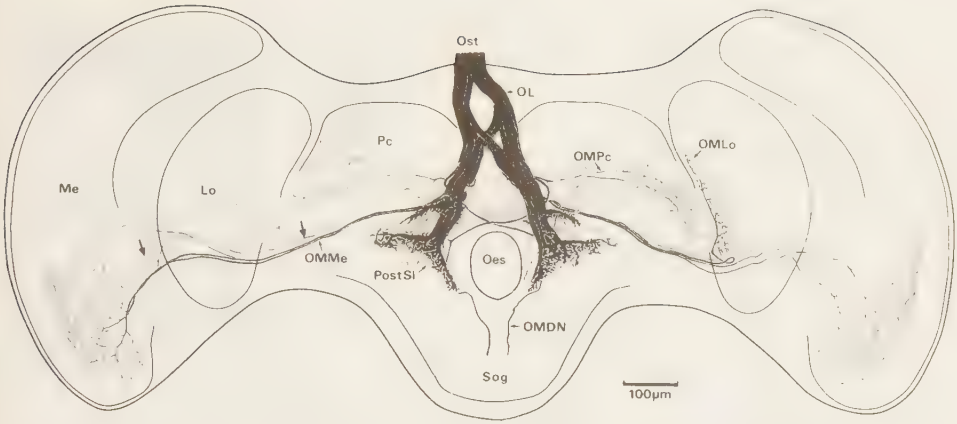

Fig. 2. Tracing of cobalt-filled ocellar interneurones from whole-mount preparation. This tracing shows the total set of large- and medium-sized neurones (except the left lobula fibre OMLo). The large-diameter fibres (*OL*) end in the posterior slope (*Post Sl*). The medium-diameter fibres project to the medulla (*Me*), the lobula (*Lo*), the protocerebrum (*Pc*) and via the suboesophagal ganglia (*Sog*) to thoracic ganglia. They are here indicated by *OMMe*, *OMLo*, *OMPc* and *OMDN*, respectively. *Arrows* indicate one thin-diameter fibre running to the medulla. *Ost* ocellar stalk; *Oes* oesophagus. In these and all other figures dorsal is up when not otherwise stated; the neurones are seen in frontal (transverse) views

interneurones underlie median and lateral ocelli respectively. Another drawback with our labelling experiments is that only the central portions of the ocellar interneurones can be resolved. For the morphology of entire neurones we have to await single cell labelling. In a number of flies ocellar interneurons with aberrant morphologies or projection patterns were encountered. These were not systematically analysed (see Goodman 1974, 1976, 1977) and will therefore not be described in this account.

Neurones with large-diameter axons (OL-neurons)

The 12 neurones with large-diameter axons (OL-neurons) have their cell bodies in the ocellar nerve (see Fig. 9) and follow the ocellar tract posteriorly within the brain. The OL-neurones separate into two groups, containing six neurons each, which terminate unilaterally on each side of the oesophagus in the bilateral posterior slope region (Figs. 2, 3a, b). On their route through the brain the 12 neurones pass dorso-posteriorly over the protocerebral bridge. Posterior to the protocerebral bridge four neurones cross over to the contralateral posterior slope. Since the morphology of the individual neurones in the peripheral ocellar neuropil has not been analysed, the connotations 'ipsi-' and 'contralateral' here refer to whether or not the neurones cross the midline of the brain. In the posterior slope region three separate termination areas can be recognised (see also Chappell et al. 1978): 1) the dorsolateral, 2) the ventrolateral, and 3) the ventro-medial (Fig. 4). The 12 OL-neurones are classified into four types according to their arborisation patterns in these areas of the posterior slope. For further classification we distinguish ipsilateral from contralateral axons. Hence, six types of OL-neurones can be distinguished, OL1-OL6 (Fig. 4). There is one bilateral pair of each type.

1. Type-OL1 neurones are ipsilateral and have terminal processes in the dorsolateral and ventro-medial area of the posterior slope. There are some spines along the axon between these areas.

2. OL2 neurones have a morphology similar to OL1, but are contralateral. Their axons cross over in a region dorsal to the oesophageal foramen (see Figs. 2, 4).

3. OL3 neurones are ipsilateral and terminate in the dorsolateral region of the posterior slope. There are some thin processes that extend into the ventro-lateral region (Fig. 4).

4. OL4 neurones are also ipsilateral. They terminate in the ventro-lateral region of the posterior slope. There are some processes reaching into part of the ventro-medial termination region.

5. OL5 neurones comprise the second type of contralateral neurones; these were never labelled alone. Therefore the exact terminal morphology was obscured by other neural elements. However, the terminations of the OL5 neurones resemble those of the OL4 neurones.

6. OL6 neurones are ipsilateral and have processes in the dorsolateral and ventro-lateral termination areas. Like OL4, some processes seem to invade the ventro-medial area. Attempts to label ocellar interneurons of the individual ocelli (medial or lateral) were not sucessful. Normally most of the large fibres (OL1-OL6) were labelled both after lesions in median or lateral ocelli.

Neurones with medium-diameter axons (OM-neurons)

The 10 neurones with medium-diameter axons project to four different areas of the central nervous system. Two neurones terminate in each medulla (Fig. 2), one neurone terminates dorsally in each lateral protocerebrum (Figs. 2, 5), one neurone ends basally in each lobula (Fig. 2), and one pair of neurones projects though the cervical connective to the thoracic ganglia (Fig. 6). The detailed morphology of these neurones is as follows.

There is one pair of medium sized ocellar interneurones

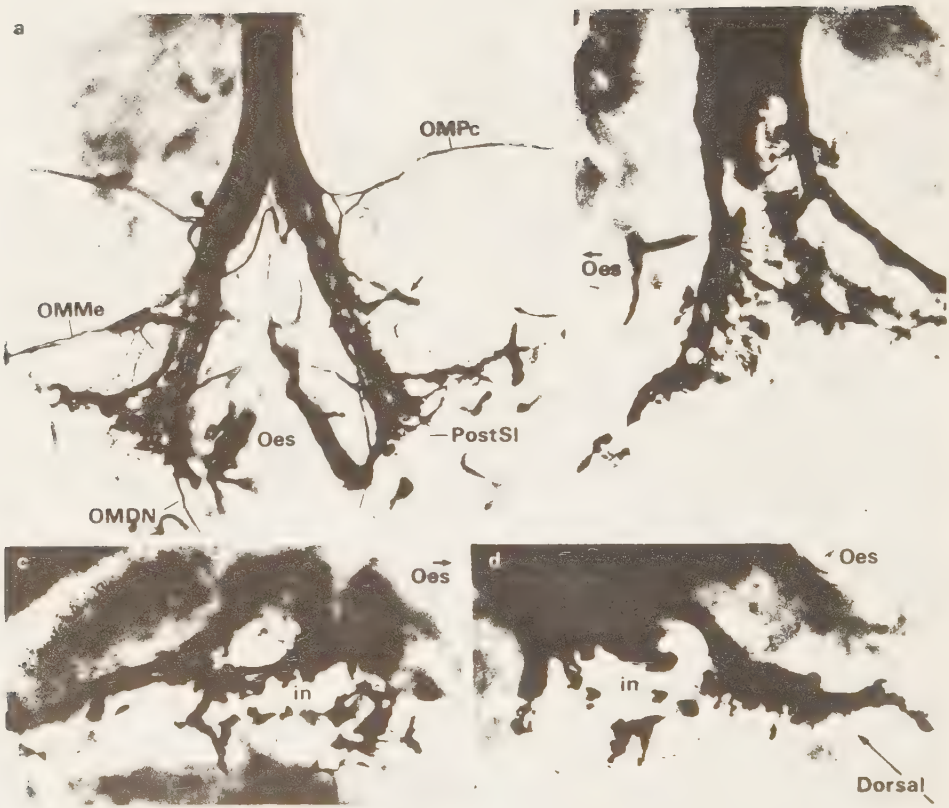

Fig. 3a. Cobalt-filled ocellar interneurones from whole mounts. Large-diameter fibres end in posterior slope (*Post SL*) on both sides of oesophagus (*Oes*). Medium-diameter fibres are indicated *OMMe*, *OMPc* and *OMDN* (cf. Fig. 2) The *arrow* points at the terminal process of an OL fibre in the PL1 termination area. X220. **b** Termination of large-diameter fibres in the posterior slope. HRP-filling, 25 µm thick section. *Oes* oesophagus. × 375. **c, d** Detail of HRP-labelled OL-fibre terminals in the posterior slope. The processes of the terminals enwrap other interneurones (*in*). *Oes* oesophagus. × 590

(OMMe) running into each ipsilateral medulla. These axons follow the central ocellar tract, join the posterior optic tract and enter the medulla, where they form large arborisations in the serpentine layer (Figs. 2, 7c). A branching process from each neurone is sent into the OL-neuron termination area in the posterior slope (Fig. 7a). In the medulla the OMMe-neurones arborise only in the ventral third of the neuropil (both anteriorly and posteriorly).

The pair of type-OMPc neurones pass the protocerebral bridge dorsally. Their axons give rise to ipsilateral, profuse branches in several regions of the lateral protocerebrum (Figs. 2, 5, 7b, d, e). Some of their processes run just below the calyces of the mushroom bodies and appear to enter the antennoglomerular tract (Fig. 7d, e) Other processes invade the lobula neuropil (Figs. 5, 7e).

On each side of the brain there is one fibre (OMLo) that joins the OMMe neurones in the posterior optic tract. These fibres then run dorsally to invade the basal part of the lobula neuropil (Fig. 2). Here they form short spiny processes in a layer close to the midbrain. Also the OMLo neurons have processes in the posterior slope.

One pair of ocellar interneurones (OMDN) has been traced down the cervical connective to the thoracic ganglia. The axons diverge from the ocellar tract posterior to the protocerebral bridge, make a loop around the central body, cross contralaterally immediately dorsal to the oesophageal foramen and run along the oesophageal foramen through the posterior slope (Fig. 6). In the posterior slope each fibre has a set of branches invading the ventro-lateral termination area (Fig. 6). The main axons proceed through the cervical connective to the dorsal parts of the pro- and mesothoracic ganglia. The arborisations of the OMDN-neurones are located in the posterior medial portion of these ganglia (Fig. 6). In the prothoracic ganglion occasionally large motorneurones contiguous with the OMDNs were transneuronally filled with cobalt.

Fig. 4. Tracing of the different types of OL-neurones, *OL1–OL6*, from cobalt fillings (whole-mount preparations). The lower part of the figure displays schematically the pattern of projection and termination in the brain. Three regions can be distinguished in the posterior slope: *I* dorso-lateral, *II* ventro-medial, *III* ventro-lateral region. OL1, OL3, OL4 and OL6 do not cross over contralaterally within the brain, whereas OL2 and possibly OL5 do. The morphology of the OL5 terminal processes is not clear since they were never labelled alone. *Oes* oesophagus

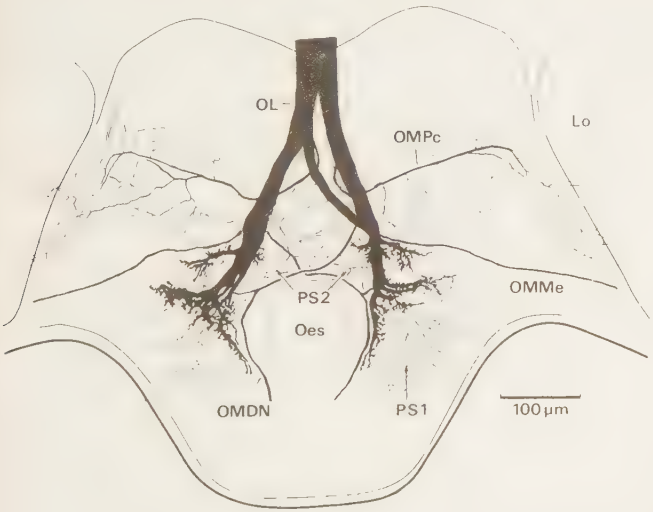

Fig. 5. Tracing from cobalt filling of ocellar interneurones (whole mount). Note rich arborisations of the OMPc neurones in the protocerebrum. Some branches also invade the lobula (*Lo*). Some of the groups of small-diameter fibres can be seen, *PS1* ventral to the posterior slope and *PS2* dorsal to the oesophagus (*Oes*)

Ocellar neurones with small diameters (OS-neurones)

Although there is no problem to recognise small fibres (OS-fibres) in transverse sections of the ocellar stalk (Fig. 1a), they are not so easy to resolve in the brain. This is mainly due to the fact that the OS-fibres follow the trajectories of OL- and OM-processes. We could, however, trace fibres of small neurones into several areas where they formed diffuse arborisations (see Fig. 9). OS-fibres accompany the OM-fibres into the medulla (Fig. 2) and the lobula (via the posterior optic tract). There are processes of thin fibres in a region dorsal to the oesophagus (Fig. 8c, d), in the posterior slope (Fig. 8b), and in a region ventral and anterior to the posterior slope (Fig. 8a). All these fibres appear varicose and spiny.

In sections with silver-intensified, cobalt-filled ocellar

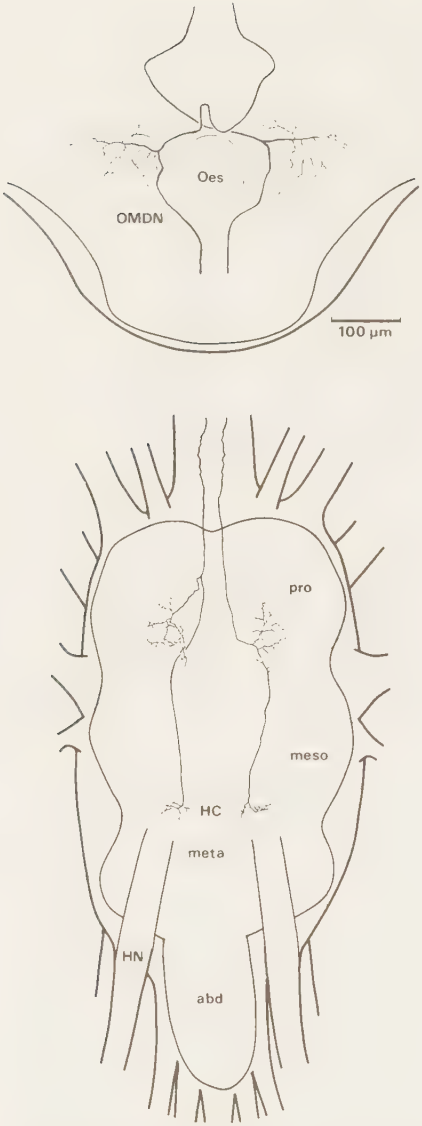

Fig. 6. Tracing of cobalt-filled descending ocellar interneurones OMDN (whole mount). The neurones cross over dorsal to the oesophagus (*oes*) and form processes in the posterior slope. In the thoracic ganglia the neurones have processes dorsally in the pro- and meso- thoracic ganglia. The posterior processes are located close to the crossing over of haltere interneurones (*HL*) *HN* haltere nerve; *Meta* metathoracic ganglion; *abd* abdominal ganglia. The thoracico-abdominal ganglia are seen in horizontal view

interneurones small-diameter fibres were seen in some additional protocerebral regions: the protocerebral bridge, the ellipsoid and fan-shaped bodies and noduli of the central body complex, a bilateral pair of small neuropils lateral to the central body, and defined regions along the entire dorsal rim of protocerebrum. The same pattern of labelled fibres was reproducable in many specimens. The labelling may, however, represent uptake through extracellular leakage or transneuronal passage of cobalt rather than primary filling of first-order ocellar interneurones. It should be noted that transneural labelling with cobalt (Strausfeld and Obermayer 1976) or HRP (Nässel 1981) did not otherwise occur in the brain.

Discussion

Connections of ocellar interneurones in different projection areas

Ocellar interneurones were found to project to at least seven main regions of the CNS (Fig. 9). Some of these are invaded by OL-, OM- and OS-fibers, others by OM- and OS-fibers, and finally some by OS-fibers or OM-fibers alone. The projection areas are: 1) posterior slope (three subregions), 2) medulla, 3) lobula, 4) pro- and mesothoracic ganglia, 5) dorsal and lateral protocerebrum, 6) a region dorsal to the oesophageal foramen, 7) areas antero-ventral to the posterior slope.

OL-neurones

The large ocellar interneurones (OL-neurones) terminate in the posterior slope. Two subregions of posterior slope, the ventro-lateral and ventral-medial termination areas, coincide with the dendritic arbors of sets of descending neurones connecting the brain to thoracic ganglia (Strausfeld 1976; Strausfeld et al. 1984). One set of descending neurones in this region appears to receive inputs both from OL-neurones and some large neurones of the optic lobes (Strausfeld et al. 1984). These lobula plate neurones are subsets of the large vertical, motion-sensitive cells (Pierantoni 1976; Hausen 1983; Strausfeld et al. 1984). Hence, there is a convergence between large diameter ocellar and optic lobe interneurones onto large-diameter neurones descending into thoraic centers.

It is apparent that the terminations of the OL-neurones in the ventral portion of the posterior slope may interact also with other interneurones in different combinations. The remaining OL-terminations and processes in the dorsolateral termination area coincide with the posterior optic tract. This tract comprises many contralateral (bilateral) visual interneurones (Strausfeld 1976) some of which may receive ocellar inputs. The separation of the posterior slope into three termination areas suggests that outputs from OL-neurones may diverge onto at least three functionally separate pathways.

OM-neurones

It is not clear whether OM-neurones are afferents projecting to central regions or efferents carrying signals to the ocellar neuropil. We can only propose that they interact with neurones in certain regions of the CNS. OM-neurones project to protocerebral centers (OMPc), the medulla (OMMe), the lobula (OMLo), and the thoracic ganglia (OMDN). The three latter types in addition have processes in the posterior

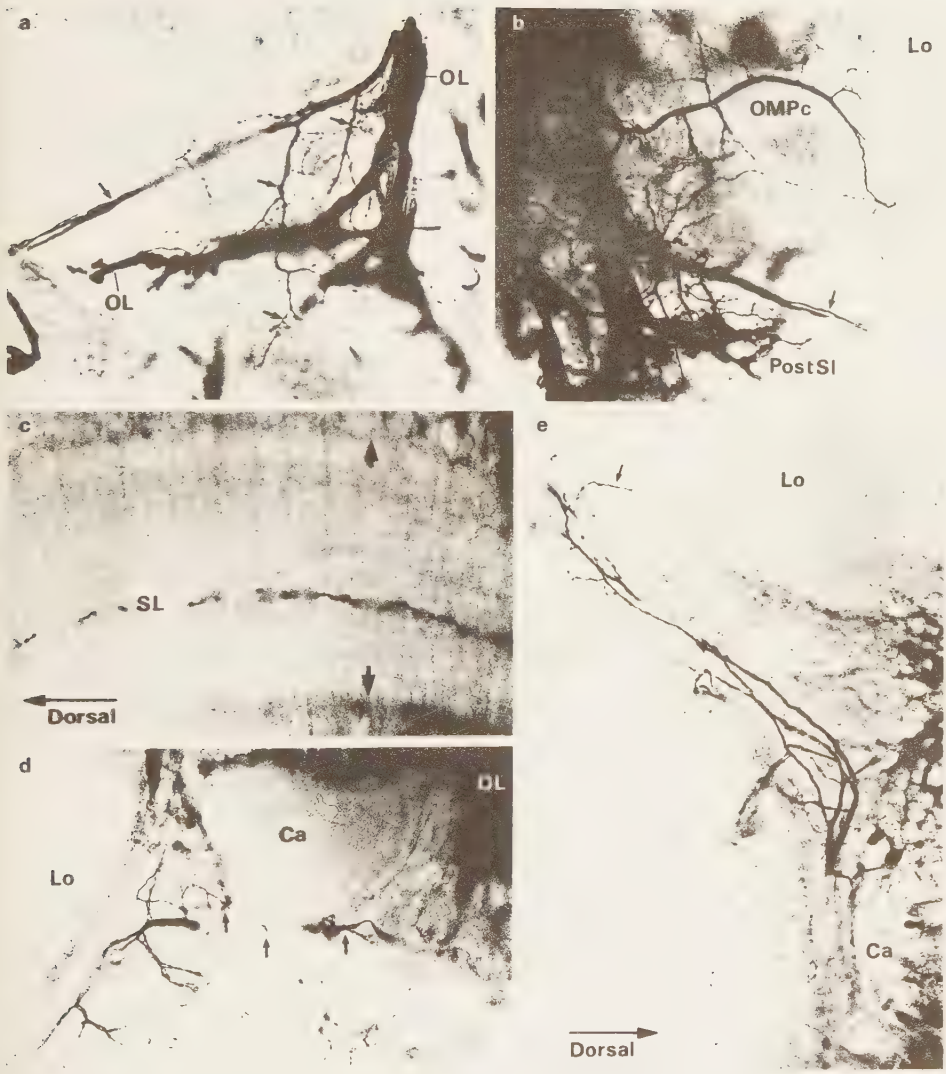

Fig. 7a–e. Cobalt filled OM-fibres from 25-µm frontal sections (except b, which is from a whole mount). **a** OMMe fibres (*arrows*) The two fibres send processes to the posterior slope, where large-diameter fibres (*OL*) terminate. ×345. **b** The process of an OMPc fibre in protocerebrum. OL fibres end in the posterior slope (*PostSL*). At *arrows* the two OMMe fibres are seen. *Lo* lobula. ×235 **c** Part of the medulla with OMMe fibres in the serpentine layer (*SL*). The *arrows* delineate the outer and inner surface of the medulla ×385. **d** Processes of an OMPc (*arrows*) run below the calyx (*Ca*) of the mushroom body. *Lo* lobula; *OL* large diameter fibres. ×235. **e** Processes of an OMPc running at the margin of the protocerebral neuropil. One process invades the lobula (*Lo*) at *arrow* The OMLo neuron of the lobula is in an adjacent section. *Ca* calyx of mushroom body. ×345

slope. Here these may interact with descending neurones (as described for OL-neurones or other sets), with OL-neurones or local interneurones.

The OMPc-neurones form extensive branches in many regions of the protocerebrum: in unstructured neuropil ventral to the calyces of the mushroom bodies, neuropil adjacent to the lobula, in the basal lobula, and possibly in the antenno-glomerular tract (AGT) (connecting antennal glo-

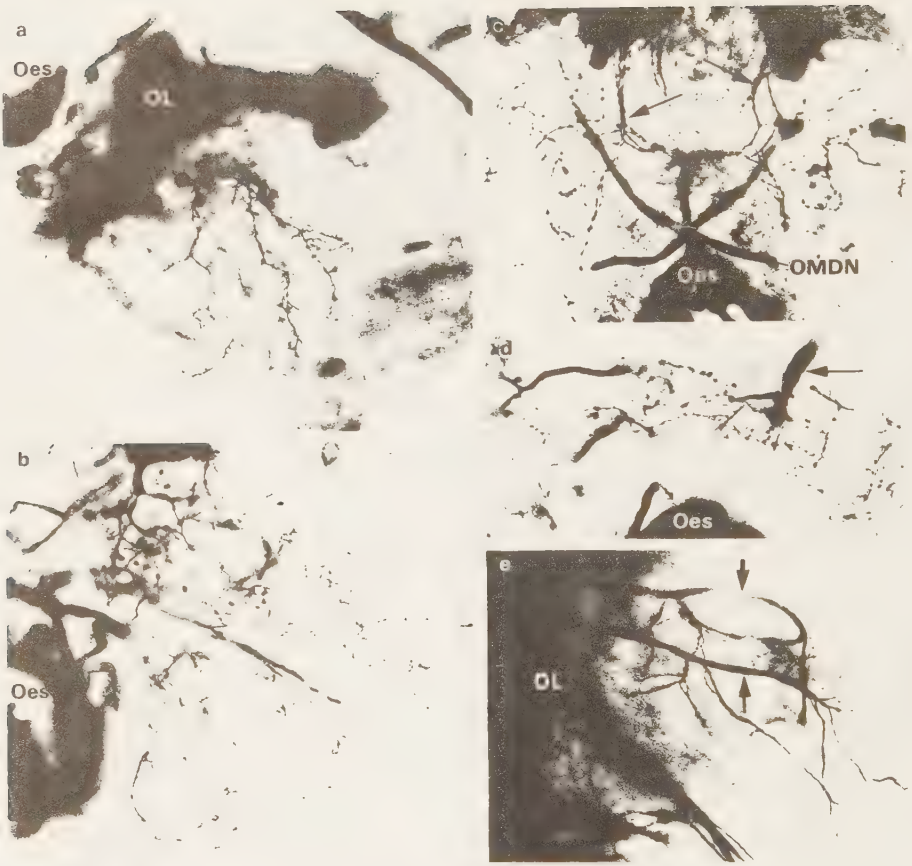

Fig. 8a–e. Cobalt-filled small-diameter fibres (OS-fibres) from 25-µm sections (except e, which is from whole mount). *Oes* oesophagus **a** Processes from OS-fibres form a plexus ventral to posterior slope (with terminals of OL-fibers) ×385. **b** OS-fibres invading the posterior slope region ×385. **c** Two bundles of OS-fibres (*arrows*) from the ocellar stalk branch form a "commisure" dorsal to the oesophagus. Some processes run ventrally towards posterior slope. Note OMDN-fibres (see also d) ×345. **d** Processes of the OS-fibres in 8b form a small plexus dorsal to the oesophagus; this plexus is termed PS2 (see Fig. 9) *Arrow* points to an OMDN-fibre. ×560 **e** This specimen of *C. erythrocephala* displays two sets of OMPc-fibres (*arrows*). This is one of many aberrant ocellar interneuron projections encountered. ×235

meruli and calyces). The OMPc-processes may thus interact with various higher order interneurones of the protocerebrum, optic neurones of the lobula and possibly chemosensory ones in the AGT. The functional organisation of much of the projection areas of the OMPc-neurones is little known; the midbrain centres of the protocerebrum may represent multimodal control centers with outputs onto descending neurones and neurones of the autonomic nervous system (Strausfeld 1976; Strausfeld and Bacon 1983; Strausfeld et al. 1984)

The OMMe- and OMLo-neurones presumably interact with the optic lobe interneurones in the medulla and the lobula, respectively. An interesting feature of the OMMe-neurones is that they arborise only in the ventral third of the medulla, and hence the ventral portions of the projected retinal mosaic. This is not the case in other studied insects (Goodman and Williams 1976; Goodman 1981; Koontz and Edwards 1984). In *C. erythrocephala* the two OMMe-neurones subserve separate (but overlapping) regions of the ventral projected mosaic (one more dorsal than the other) as seen in Fig. 2. There thus appears to be an interaction between the dorsal visual fields subserved by the ocelli and a ventral one viewed by the ventral part of the compound eyes. In the lobula the interaction with the OMLo-neurones may involve the entire projected visual field.

The OMDN-neurones connect the ocellar neuropil to

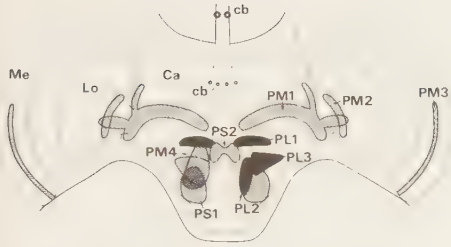

Fig. 9. Summary diagram of projection areas of ocellar interneurones. OL-neurones invade three regions of the posterior slope *PL1–PL3*. OM-neurones invade protocerebrum *PM1*, the lobula *PM2*, the medulla *PM3*, posterior slope *PM4*, and the thoracic ganglia (not shown). Os-neurones invade a region anterio-ventral to posterior slope (*PS1*) and a neuropil dorsal to the oesophagus (*PS2*). They also accompany OM neurones in most regions (not indicated in this Fig.). *Me* medulla; *Lo* lobula; *Ca* calyx of mushroom body; *Cb* cell bodies (large circles indicate the 12 cell bodies of L-fibres, small circles the 10 cell bodies of M-fibres)

the posterior slope and pro- and mesothoracic ganglia. The processes in the thoracic ganglia are dorsal, but not located in flight motor centers. The prothoracic processes may contact large motorneurones probably running to neck muscles; the mesothoracic processes lie at the border with the metathoracic ganglion where the sensory axons of the halteres connect to large interneurones.

OS-neurones

OS-neurones form three termination areas of their own: two bilateral-symmetrical anterio-ventral to the posterior slope (PS1) and one fused medial dorsal to the oesophageal foramen (PS2). The PS1 area may partly overlap with endings of antennal mechanosensory fibres (Strausfeld 1976; Nässel et al. 1984) and extend partly into the tritocerebrum. Varicose fibres in the PS2 area may interact with fibres running in a posterior commisure. The remaining OS-fibres invade areas also innervated by OL- and OM-fibres such as posterior slope and the medulla.

In addition, it appears as if some of the OS-fibres arborise in the ocellar stalk. We have not yet performed an extensive electron-microscopical study to reveal whether synapses are formed within the ocellar stalk (cf. Goodman 1981; Koontz and Edwards 1984). Some OS-fibres may be efferents with outputs in the peripheral ocellar neuropil or the ocellar stalk.

Comparison with other insects: anatomy and function

Since electrophysiological studies involving the ocellar system have been conducted mainly in insects other than the blowfly a discussion concerning functional aspects requires a comparison with other species.

In the insects studied many ocellar projection areas are in common. In bees, locusts, crickets and cockroaches the following projection areas are found (reviewed by Goodman 1981; Koontz and Edwards 1984): posterior slope, protocerebral bridge, medulla (not in locusts), lobula, antenno-glomerular tract, ocellar tract (not in bees). Except for the protocerebral bridge and possibly the ocellar stalk the ocellar interneurones of *Calliphora erythrocephala* arborise in the same areas. Additional areas are found in some other insects as well as in *Calliphora*: ventral bridge and tritocerebrum (PS1 in *Calliphora*) in locusts (Goodman and Williams 1976), a moth (Eaton and Pappas 1978) and the bee (Goodman 1981); neuropil near the posterior optic tract (PS2 in *Calliphora*) in cricket and cockroach (Koontz and Edwards 1984). First-order ocellar interneurones to thoracic ganglia (OMDN in *Calliphora*) are seen in bees, dragonflies (Goodman 1981) and desert ants (Meyer personal communication). These interneurones were also resolved after injection of Procion yellow in *Calliphora* (Hengstenberg and Hengstenberg 1980).

In all studied insects the large-diameter axons terminate in the posterior slope (in bees some continue to thoracic ganglia). There is a large variation in the number, morphology and trajectories of OL-neurones among insects. In most studied insects individual interneurones from the median ocelli may project to one side of the brain or both or run back to a lateral ocellus; from lateral ocelli the axons may be ipsilateral, contralateral or project back to the median ocellus (reviewed by Goodman 1981). In a Golgi-impregnation study of *Musca domestica* Strausfeld (1976) found that of the 12 OL-neurones, three pairs arborise beneath a single ocellus (two to each), four innervate pairs of ocelli, and one pair subserve all three ocelli.

In *Calliphora erythrocephala* and other studied insects the OL-fibres may relay input onto descending neurone systems used in control of flight and head positioning (Stange 1981; Taylor 1981; Wilson 1981; Rowell and Pearson 1983) These descending pathways have convergent multimodal inputs (cf. Goodman 1981; Simmons 1981b; Rowell and Pearson 1983; Strausfeld and Bacon 1983; Strausfeld et al. 1984). In flies and locusts there are, for instance, convergences between large ocellar interneurones and motion-sensitive, optic lobe neurones (Strausfeld et al. 1984; Simmons 1981a).

The detailed morphology of OM- and OS-fibres also varies in insects. One main difference is that *C. erythrocephala* appears to lack first-order innervation of the protocerebral bridge and mushroom body calyces; we found no candidates for centrifugal (efferent) neurones (cf. Goodman 1981; Koontz and Edwards 1984). The ocellar inputs relayed by medium- and small-diameter neurones seem to provide multimodal interactions in various areas: optic lobes, mechanosensory centers, posterior slope, unstructured protocerebral neuropil, mushroom bodies (possibly chemosensory interneurones). The function and complexity of these interactions are yet to be elucidated.

In conclusion, our data show that the ocellar pathway in *Calliphora erythrocephala* in general resembles that of bees, locusts, crickets and cockroaches. Differences that may be of functional significance lie in the organisation of OL-neurones in relation to the individual ocelli and the number and profusion of branches of medium and small ocellar interneurones. Although the termination areas of ocellar interneurones are known, the detailed connections of these and other neurones remain to be studied.

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III

Interneurones subserving ocelli in two species of trichopterous insects: Morphology and central projections.

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SUMMARY

The central projections of ocellar interneurones in two trichopterous insects Agrypnia varia F. and Limnephilus flavicornis F. were analysed using cobalt iontophoresis. The interneurones were classified into three groups; large-, medium- and small-diameter neurones based on the diameters of the axons.

Seven large-diameter neurones project from each lateral ocellus into the central nervous system. Of these, four neurones terminate in the posterior slope (three ipsilateral and one contralateral). Three neurones possess branches in the contralateral posterior slope and proceed down the cervical connective into the thoracic ganglia. Medium-sized neurones connect the neuropiles of the three ocelli to each other. Small diameter neurones contact the contralateral lobula and medulla of the optic lobes and connect the three ocellar neuropiles. Large-diameter neurones of the median ocellus were found to terminate bilaterally or ipsilaterally in the posterior slope.

In the posterior slope four different subregions can be recognized: 1) the dorso-lateral, 2) the ventro-lateral and 3) the lateral, into which large diameter interneurones of the lateral ocelli send branches, and 4) the medial, innervated by interneurones of the median ocellus. Interneurones of the median ocellus send branches into the lateral region as well.

Key words: Insect visual system - Ocellar pathway - Visual interneurones - Neuronal tracing - Agrypnia varia - Limnephilus flavicornis .

INTRODUCTION

Different functional roles have been ascribed to the ocelli of insects, such as producing muscular tonus to the flight system, flight equilibrium control (Stange 1981; Stange and Howard 1979; Wilson 1978; Rowell and Pearson 1983), orientation in walking flies (Wehrhan 1984) and ants (Fent and Wehner 1985) and possibly in the dorsal light response used during flight (Kalmus 1945; Goodman 1965). The interneurones of the ocellar system have been studied in insects representing the orders Blattoidea, Orthoptera, Lepidoptera, Hymenoptera and Diptera (For references, see Goodman 1981; Koontz and Edwards 1984; Nässel and Hagberg 1985). The present report is a study of the ocellar interneurones in two insects, Agrypnia varia F. and Limnephilus flavicornis F. belonging to the families Phryganeidae and Limnephilidae, respectively, of the holometabolous order Trichoptera. The flight activities of these species are restricted to dusk and dawn, differing from other species where the ocellar interneurones have been traced: the cockroach and the moth are nocturnal; crickets, locusts, bees, wasps and the blowflies are diurnal insects flying in reasonably bright light, and dragonflies are active in high ambient light-intensities. The difference in flight activity and complexity of flight behaviour between these insects might be reflected in the organisation of the ocelli and the ocellar pathway. Thus, a comparison between the ocellar pathways of previously studied insects and trichopterans may be of value. In this context it should be noted that some Trichoptera totally lack ocelli or at least external ocelli (Ross 1967). In the present report the pathways, the areas of termination and the morphology of the interneurones of the three dorsal ocelli are described based on tracings

after cobalt filling. A comparison with other insects is also made and it can be concluded that the two species studied have an ocellar pathway as complex as that of the visually oriented bees and flies.

MATERIALS AND METHODS

The trichopterous insects (caddies flies) Agrypnia varia F. and Limnephilus flavicornis F. were collected at Stensoffa ecological station 20 km east of Lund by using a mercury lamp. The median or one lateral ocellus of Agrypnia and Limnephilus were filled with $CoCl_2$. The analysis of the ocellar interneurones is based on 25 successful fillings of one lateral ocellus and 15 successful fillings of the median ocellus of each species. The insect was fixed with wax onto a glass slide with the head immobilised. The ocellar lens was cut off with a razor blade, the underlying neuropile was pierced with a thin needle, and $CoCl_2$ crystals were applied. The cut was sealed with vaseline. The preparations were left for 12h at 4°C for cobalt uptake. The animals were decapitated and the opened heads were immersed in ammonium sulphide (10 drops to 20ml distilled water) for 10 min. The dissected brains were fixed for 3h in acetic acid-alcohol-formalin fixative and silver intensified according to Bacon and Altman (1977). The cobalt-labelled tissue was embedded in Araldite and frontal sections were cut at 25µm. The tracings of cobalt-labelled neurones were made with a drawing tube fitted to a Leitz Orthoplan microscope using a x63 planapochromate oil immersion lens.

RESULTS

The two trichopterous species Agrypnia varia F. and Limnephilus flavicornis F. have three distinct and well-separated ocelli. Two ocelli are situated laterally with lateral fields of view while the third ocellus is located medially, situated between the antennae, and has a frontal field of view (Hallberg and Hagberg in preparation). Exteriorly the ocelli are well developed with prominent lenses. A.varia has a biconvex cuticular lens whereas in L. flavicornis the convexo-concave cuticular lens is thin and supplemented by an underlying liquid-filled space. The arrangements of the retinular cells, rhabdoms and the tracheal sleeve around the ocelli are similar in the two species (Hallberg and Hagberg, in preparation). The ocellar photoreceptors end in a peripheral neuropile. Each neuropile is connected to the brain by means of a short ocellar nerve. In the median ocellus the nerve and the distal neuropile are divided into two portions indicating a paired origin.

Neuronal projections from the lateral ocelli of Agrypnia varia F.

Ocellar interneurones with large diameter (LOL-neurones).

Four large-diameter neurones project to the posterior slope of the brain (Figs.1A,B,2A,3A,C). Three of these neurones end in the ipsi-lateral posterior slope, while the fourth neurone crosses over inside the brain to project to the contralateral posterior slope. The crossing over takes place at the level of the posterior optic tract. These four neurones constitute the most dorso-posterior projection of the lateral ocellar

interneurones in the posterior slope. This area is situated close to the posterior optic tract (Fig.2A). The cell bodies reside dorsal to the terminations of the fibers (Fig.1B; see also 3D). The axons of the large-diameter neurones have a diameter of ca. 10µm and their cell bodies have a diameter of ca. 8µm. The neurones are termed LOL 1-4 (lateral ocellus large-diameter neurones).

The LOL 1-3 neurones terminate in the ipsi-lateral posterior slope. LOL 1 has the most lateral projection of these three ipsilaterally projecting neurones. The LOL 2 neurone terminates more medially compared to the LOL 1 neurone. The LOL 3 neurone is the most medially situated of the three ipsilaterally projecting neurones. Its arborisation pattern is similar to LOL 1 and LOL 2 but less extensive. There is a slight overlap laterally between the terminal branches of LOL 1-3. The LOL 4 neurone terminates in the contralateral posterior slope. The neurone has a bifurcated appearance in the posterior slope and a large lateral extension, which equals that of the LOL 1-3 neurones from the contralateral ocellus. Without differential labelling of the interneurones in the two lateral ocelli it is, howewer, not possible to determine whether there is any overlap between terminations of LOL 4 and LOL 1-3.

Three large-diameter descending neurones project to the thoracic ganglia, termed LOLDN 1-3 (lateral ocellus large-diameter descending neurones) (Figs.1A,C,2A,3E). The axons of the three neurones cross over dorsal to the oesophagal foramen to project down the contralateral oesophageal connective into the suboesophageal ganglion. Here, short, stout branches are formed by all three neurones (Fig.1A,C) and the main fibres proceed down the cervical connective. Two of the neurones have extensive partially overlapping arborisations in the posterior slope

(Figs.1A,C,2A,3B,E). The projectional pattern in the thoracic ganglia has not yet been mapped due to problems with incomplete cobalt-filling over the long distance involved.

The LOLDN 1 neurone has the largest diameter (c. 10um). It runs along the path of the LOL 1-4 neurones and crosses over together with the LOL 4 neurone. A large branch is sent into the dorso-lateral posterior slope area of termination. This branch splits into several secondary branches, the arborisations of which mingle with those of the contralaterally projecting LOL 4 neurone. Several additional branches from the main axon pass into the ventro-lateral posterior slope. The LOLDN 2 neurone has a smaller diameter (ca. 8μm) and runs along the LOLDN 1 neurone. The neurone sends off a few thick branches into the posterior slope. The main processes from these branches are in the ventro-lateral posterior slope. The axon of the LOLDN 3 neurone runs more anteriorly within the dorsal part of the brain than the other LOLDN neurones. Some small branches are sent off into the ventro-lateral posterior slope before the main axon enters the oesophageal connective.

Ocellar interneurones with small (LOS-neurones) and medium diameters.

Three neurones with small-diameter axons project to the contralateral lobula and medulla of the optic lobe (Figs.1,4). They all arborise in the basal neuropile of the lobula and medulla (Fig.4). The axons have a diameter of ca. 2μm and in addition arborise in the lateral protocerebrum. Some of these branches send processes into the posterior optic tract (Figs.1,2A). The neurones are termed LOSOL 1-3 (lateral ocellus small-diameter optic lobe neurones). One small neurone, termed LOSPc (lateral ocellus small-diameter protocerebral neurone), was found that runs to the contralateral dorso-lateral protocerebrum (Figs.1A,2A,

3F). When filling the right ocellus, bundles of small diameter fibres can be traced to the left and median ocellar neuropile (Fig.2A). Their cell bodies are situated dorsally in the protocerebrum below the median ocellar nerve. The fibres arborise in the peripheral neuropile of the three ocelli. As described in more detail below three neurones with medium-diameter axons connect all the three ocellar neuropiles with each other (see Fig.5A).

Neuronal projections from the median ocellus of Agrypnia varia F.

Ocellar interneurones with large diameter (MOL-neurones).

The interneurones of the median ocellus consist of two pairs of large-diameter (ca. 10µm) neurones. These four neurones were never filled from the lateral ocelli. The two pairs of neurones are termed MOL 1 and MOL 2 (median ocellus large-diameter neurones).

The two MOL 1 neurones constitute one pair (Figs.5A,B). The axon of a MOL 1 runs laterally and posteriorly in the median ocellar tract to terminate in the ipsilateral posterior slope. Here, processes are sent into two areas, one medially situated, and specific for the median ocellar projection, and one laterally coinciding with the lateral area of the lateral ocellar projection. Each of the two MOL 2 neurones projects bilaterally within the posterior slope (Figs.5A,C;6). Their termination area is situated anterior to that of the MOL 1 neurones and the processes invade the lateral ocellar focus.

Ocellar interneurones with medium diameter (MOM-neurones).

Three neurones, termed MOM 1-3 (median ocellus medium-diameter

neurones), are found connecting the median ocellus to the two lateral ocelli (Fig. 5A). These three neurones (diameter ca. 4um) can be filled from the median ocellus or from either of the lateral ocelli. In the ocellar neuropiles they have thin ramifications. The MOM 3 neurone differs from the MOM 1 and the MOM 2 neurones in that the main axon in each lateral ocellar tract divides into four thin branches. These branches run along the lateral ocellar tracts to form a network of thin ramifications distally.

Neuronal projections from the ocelli of Limnephilus flavicornis F. .

The neurones projecting to the posterior slope areas and the thoracic ganglia conform in number and arborization patterns to those described above for Agrypnia varia F. and will therefore not be further commented upon. Neurones from the lateral ocelli of the two species can be compared in Fig.2. Three fibers projecting towards the contralateral optic lobes were resolved, probably corresponding to those of A. varia projecting to the lobula and the medulla. In no case, however, were the processes of these neurones in the optic lobes completely filled with cobalt in L. flavicornis. Two neurones, corresponding to LOSPc, project to the contralateral protocerebrum. The same types of small and medium fibres as in A. varia connect the ocellar neuropiles so that each ocellus is connected to the other two ocelli.

DISCUSSION

Ocellar interneurones in the two trichopterous species.

Although the anatomy of the dioptric elements in the ocellar cups of the two trichopterous species differs as described in Results (Hallberg and Hagberg, in preparation) the morphology and projections of the interneurones of the median and the lateral ocelli have been found to be very similar. The same number of large-diameter neurones projects to the posterior slope and the thoracic ganglia. The arborisation patterns of these neurones and the three small-diameter neurones projecting to the contralateral optic lobes (lobula and medulla) as well as the neurones interconnecting the lateral and medial ocellar neuropiles are similar in the two species. Although some neurones in L. flavicornis were incompletely labelled we suggest that the total set of ocellar interneurones may be similar in terms of number and projectional pattern in the two species. This assumption will also form the basis of the following discussion.

The ocellar interneurones of the lateral ocelli project to the following areas within the brain: The optic lobes (lobula and medulla), the lateral, dorsal and medial protocerebrum, the contralateral ocellus, the median ocellus, the posterior slope (which can be divided into the medial, the dorso-lateral, the ventro-lateral and the lateral ocellar focus), the posterior optic tract and finally a small area in the sub-oesophagal ganglion. Ocellar interneurones of the median ocellus have been found to project to the neuropiles of the two lateral ocelli and to the posterior slope (to the lateral and the medial ocellar foci). In

addition, descending neurones from the lateral ocelli to the thoracic ganglia have been traced. These areas are mapped in Fig.7A-C.

Comparison with other insects.

The basic pattern of projections of the ocellar interneurones is the same in those insects studied to date: first order interneurones contact the following areas in the brain: The posterior slope, the optic lobes, the lateral protocerebrum and the ocellar neuropiles. In the different insects this is done by constellations of neurones varying in number, size and arborisation pattern. In some insects ocellar interneurones project to additional areas, e.g., protocerebral bridge and antenno-glomerular tract (bees, locusts, crickets and cockroaches), ventral bridge (locusts, moths and bees), and tritocerebrum (locusts, moths, bees, blowflies and caddiesflies), in the vicinity of the posterior optic tract (cricket, cockroach and blowfly) (Eaton and Pappas 1978; Goodman 1976; Goodman and Williams 1976; Goodman et al. 1975; Pan and Goodman 1977; Pappas and Eaton 1977; Koontz and Edwards 1984; Milde 1984; Nässel and Hagberg 1985). Furthermore, first-order ocellar interneurones projecting to the thoracic ganglia have been reported in the bee (four pairs of contralaterally projecting neurones and one pair of ipsilaterally projecting neurones from each lateral ocellus and one pair of neurones from the median ocellus) (Goodman 1981) and in the blowfly (one pair of contralaterally projecting neurones (Nässel and Hagberg 1985)). The lateral ocelli of A. varia and L. flavicornis each have three pairs of contralaterally projecting descending neurones, whereas the median ocellus has no such fibres. It should be noted that the descending neurones are of large diameter in the caddiesflies, while in the blowfly and in the bee the diameter of these neurones are of medium size (diameters less than 5µm) and in many insects they appear to

be lacking. The descending neurones of the blowfly also differs in having no branches within the suboesophageal ganglia. This variation in number and diameter of axons may indicate a variation in importance of the directly descending first order interneurones between insects.

In the hemimetabolous insect orders Blattoidea and Orthoptera the large ocellar interneurones project only ipsilaterally within the brain. Here, the lack of contralaterally running ocellar interneurones is combined with less well-developed ocelli and a less pronounced division of the median ocellus (Goodman 1981; Koontz and Edwards 1984). In the holometabolous orders Diptera, Hymenoptera and Lepidoptera as well as in the hemimetabolous order Odonata ocellar interneurones from both the lateral ocelli and the median ocellus project ipsi- as well as contralaterally within the brain (Pappas and Eaton 1977; Goodman 1981; Nässel and Hagberg 1985). In trichopterans, there is an abundance of contralateral ocellar neurones from the lateral ocelli but no purely contralateral ones from the median ocellus. Instead, bilaterally projecting neurones are found subserving the median ocellus. In insects with a median ocellus there is a difference in morphology and projections between interneurones from median and lateral ocelli (Goodman 1981). This difference is pronounced in the two trichopterous insects. No descending neurones or neurones to the optic lobes originate in the median ocellar neuropile and the ipsilaterally projecting interneurones form an additional termination area not invaded by lateral ocellar interneurones.

The two trichopterous have recurrent connections between the lateral and median ocellar neuropiles in contrast to dragonflies, bees, wasps, cockroaches and moths (the latter two lack a median ocellus). Large-diameter neurones running between lateral and median ocelli have been

documented in locusts, crickets and caddiesflies. In locusts and crickets two neurones run between the median and each lateral ocellus, while in caddiesflies three neurones run between the median and both of the lateral ocelli. In the blowfly these contacts may be carried out in the fused distal neuropile by processes of the large diameter interneurones. Functionally, these interocellar neurones may modulate ocellar activity and/or integrate ocellar output (Koontz and Edwards 1984). Considering the number of neurones and neuronal types as well as profusion of fiber projections, an increasing complexity is found from cockroach to cricket to locust (Koontz and Edwards 1984). The ocellar pathways of bees, wasps, blowflies, moths and dragonflies all seem to be more complex than that of the locust. In comparison, the ocellar pathway of the two trichopterous insects seems to be at least as complex as that of hymenopterous and dipterous insects.

This complexity in the morphology of the ocellar neuronal system implies ocellar functions similar to those of, e.g., dipterous and orthopterous insects. Here, ocellar interneurones contact descending pathways with convergent multimodal inputs (Goodman 1981; Simmons 1981b; Rowell and Pearson 1983; Strausfeld and Bassemir 1985) used in control of flight and head positioning. The complexity of the posterior slope termination areas and the projection of ocellar interneurones to many other termination areas imply that ocelli feed input into many pathways. Some of these pathways may serve functions other than orientation and posture. It has also been shown that ocellar interneurones can be multimodal with inputs from other sensory systems (Milde and Homberg 1984). This indicates that ocellar interneurones may not only relay ocellar sensory information to various parts of the CNS, but may also integrate and relay other information.

The present investigation did not reveal any significant differences in the anatomy of the ocellar pathway (except in details) compared to other insects with complex ocellar systems. It, howewer, showed that the lateral and medial ocelli form distinct separate pathways and that these pathways, to be understood functionally, must be analysed physiologically and anatomically in conjunction with the neurones with which they interact.

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FIGURE LEGENDS

Fig. 1A. Semi-diagramatic tracing of cobalt-filled interneurones of the median (MO) and one lateral (LO) ocellus of Agrypnia. This and all other illustrations are from frontal sections.

Fibres project to medulla (Me), lobula (Lo), lateral protocerebrum (Pc) and to ipsilateral (PST) and contralateral (DL and VL) posterior slope.

Oes=oesophageal foramen; SOE=suboesophageal ganglia.

B. The large ocellar interneurones LOL 1-4 terminating in the posterior slope. Their cell bodies (cb) are ipsilateral. Oes=Oesophageal foramen.

C. The three descending ocellar interneurones LOLDN 1-3 with processes in dorso-lateral (DL) and ventro-lateral (VL) posterior slope and in the suboesophageal ganglia (SOG). Oes=oesophageal foramen.

Fig.2A. Tracing of cobalt-filled interneurones from a lateral ocellus in A. varia (frontal view). Three large ocellar interneurones (LOL 1-3) terminate in the ipsilateral posterior slope (PST) termination area. Another neurone terminates in the contralateral posterior slope and along the posterior optic tract (POT). Three descending ocellar interneurones (LOLDN 1-3) arborise in the contralateral posterior slope and lateral to the oesophageal foramen (Oes) in the ventrolateral ocellar focus (VL). Small ocellar interneurones run to different parts of the brain. Three neurones (LOSOL) invade the optic lobes and regions in the lateral and median protocerebrum, one neuron (LOSPc) runs to dorso-lateral protocerebrum and others connect the neuropiles of the contralateral (Lat) or median (Med) ocellus. Their cell bodies (Cb) are located dorso-medially. Some small ipsilateral neurones (asterisk) invade a region ventral to the posterior slope.

B. Tracing of some of the interneurones of the lateral ocellus of L. flavicornis (frontal view). The three large ipsilateral neurones terminate at arrow. LOL 4, the contralateral large-diameter neurone, terminates in posterior slope and posterior optic tract (POT). Also the three descending ocellar interneurones (LOLDN 1-3) are similar to these in A. varia .Only the small interneurones ending ventral to the posterior slope (asterisk) are shown. Arrow points to ipsilateral posterior slope terminals (LOL 1-2).

Fig.3. Micrographs of cobalt-filled interneurones from one lateral ocellus of A. varia ,except D which is L. flavicornis (from 25um thick frontal sections). Scales A=100µm, B-F=50µm.

A. Large ocellar interneurones terminating in the posterior slope dorsal to the oesophageal foramen (oes). One of the three descending ocellar interneurones is seen at arrow. This neurone sends a branch to the posterior slope.

B. Higher magnification of the same section showing the processes in the posterior slope and along the posterior optic tract (pot).

C. Termination of the contralateral large ocellar interneurone (LOL 4) in the posterior slope.

D. The terminations of the three ipsilateral large interneurones (LOL 1-3) in the posterior slope of L. flavicornis . Their cell bodies can be seen at arrows.

E. The branches of two descending ocellar interneurones in the region lateral to the oesophageal foramen (ventro-lateral posterior slope).

F. Processes of small ocellar interneurones (LOSPc) in dorso-lateral protocerebrum.

Fig.4. Tracings of the small ocellar interneurones (LOSOL 1-3) projecting from a lateral ocellus to the optic lobes (medulla and lobula). Note that the processes invade only the basal part of the neuropile regions.

Fig.5.Tracings of cobalt-filled neurones subserving the median ocellus (MO).

A.Two types of large-diameter fibers can be resolved, one pair of MOL 1 neurones and one pair of MOL 2. These terminate in the posterior slope (PST). The MOL 1 neurones also form terminations medially (arrow) in the

median ocellar focus. Three neurones MOM 1-3 with medium-diameter axons connect all three ocellar neuropiles. From the branches in the lateral ocellar neuropile (LON), receptor cells in the ocellar retina (OR) were filled transneuronally (only a small sample was traced). Oes=oesophageal foramen.

B. Two MOL 1 neurones.

C. One of the two bilateral MOL 2 neurones (same scale as B).

Fig.6. Micrographs of cobalt-filled interneurones from the median ocellus of A. varia. The two bilateral neurones, MOL 2, terminate in the lateral posterior slope (L). In the median posterior slope (M) some terminations from the ipsilateral MOL 1 neurones can be seen. Oes=oesophageal foramen. Scale:50 µm.

Fig.7. The termination areas of ocellar interneurones. Black areas depict terminations of large-diameter neurones, shaded areas are those of small fibers. Arrows indicate site of filling.

A. Filling of one lateral ocellus. Large fibers terminate in dorso-lateral (DL), lateral (L) and ventro-lateral (VL) posterior slope and in the suboesophageal ganglia (SOG). Small fibres terminate in protocerebrum (Pc), medulla (Me), lobula (Lo) and posterior slope.

B. Filling of median ocellus. Large fibres end in median (M) and lateral (L) foci of posterior slope.

C. Summary of projection areas of all three ocelli.

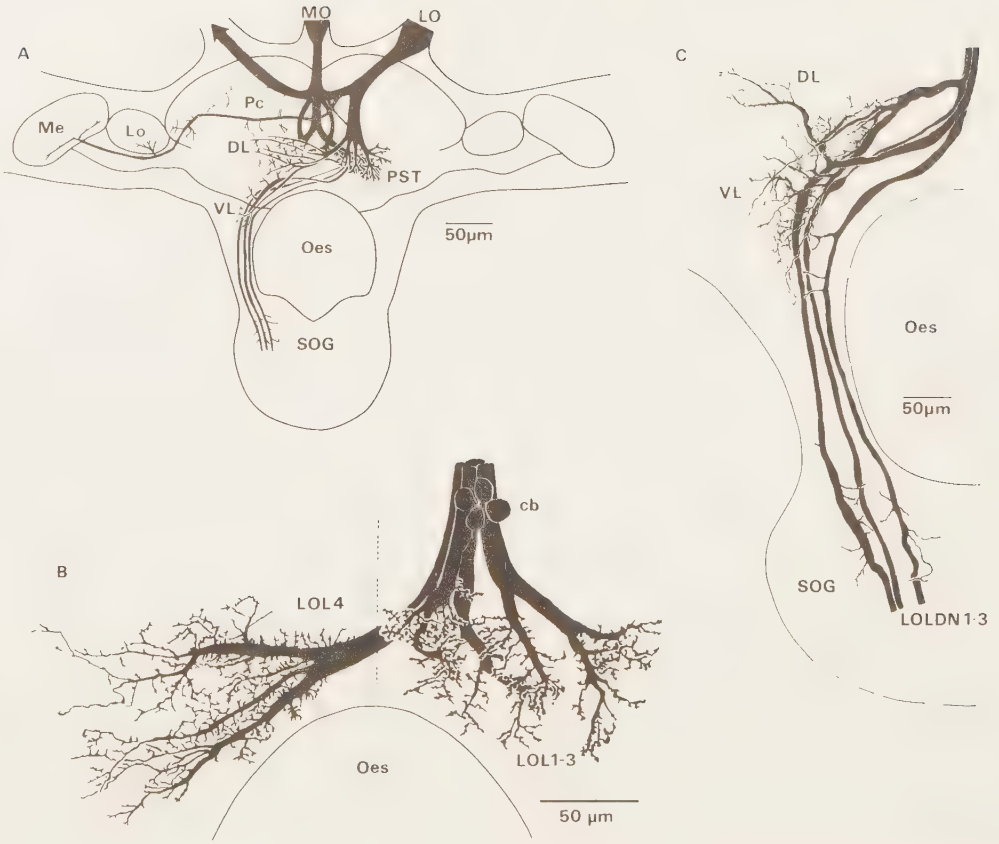
A
MO
LO
Pc
Me
Lo
DL
PST
VL
Oes
50µm
SOG
B
cb
LOL4
LOL1-3
Oes
50 µm
C
DL
VL
Oes
50µm
SOG
LOLDN1-3

Fig 1

A
Med
Lat
Cb
LOSPc
POT
PST
LOSOL
VL
Oes
50µm
LOLDN1-3
B
LOL4
POT
VL
Oes
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LOLDN
1-3

Fig 2

Fig 3

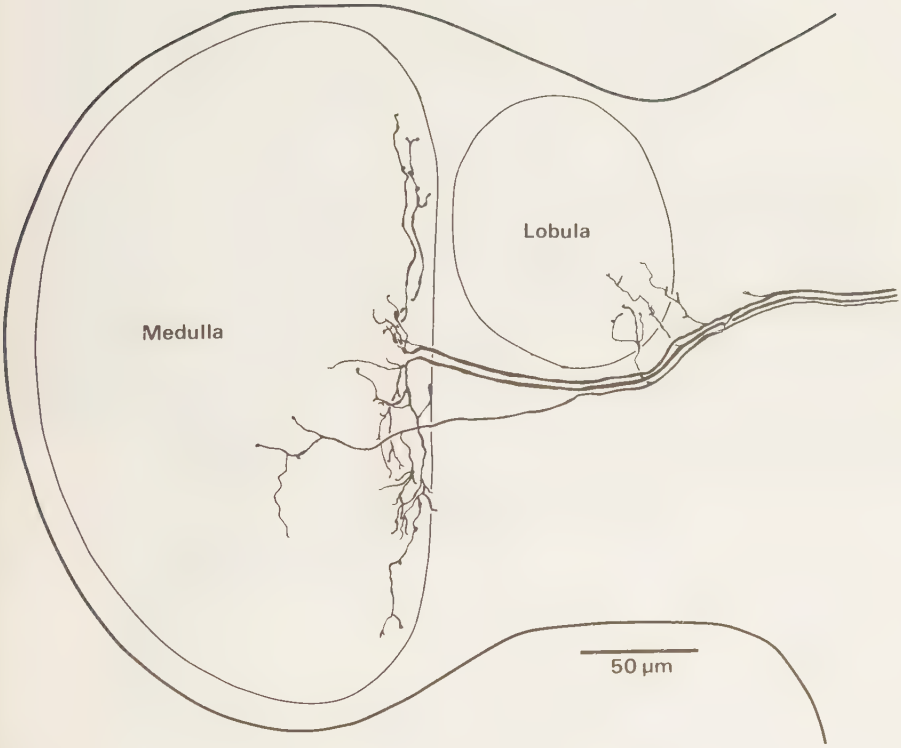

Fig 4

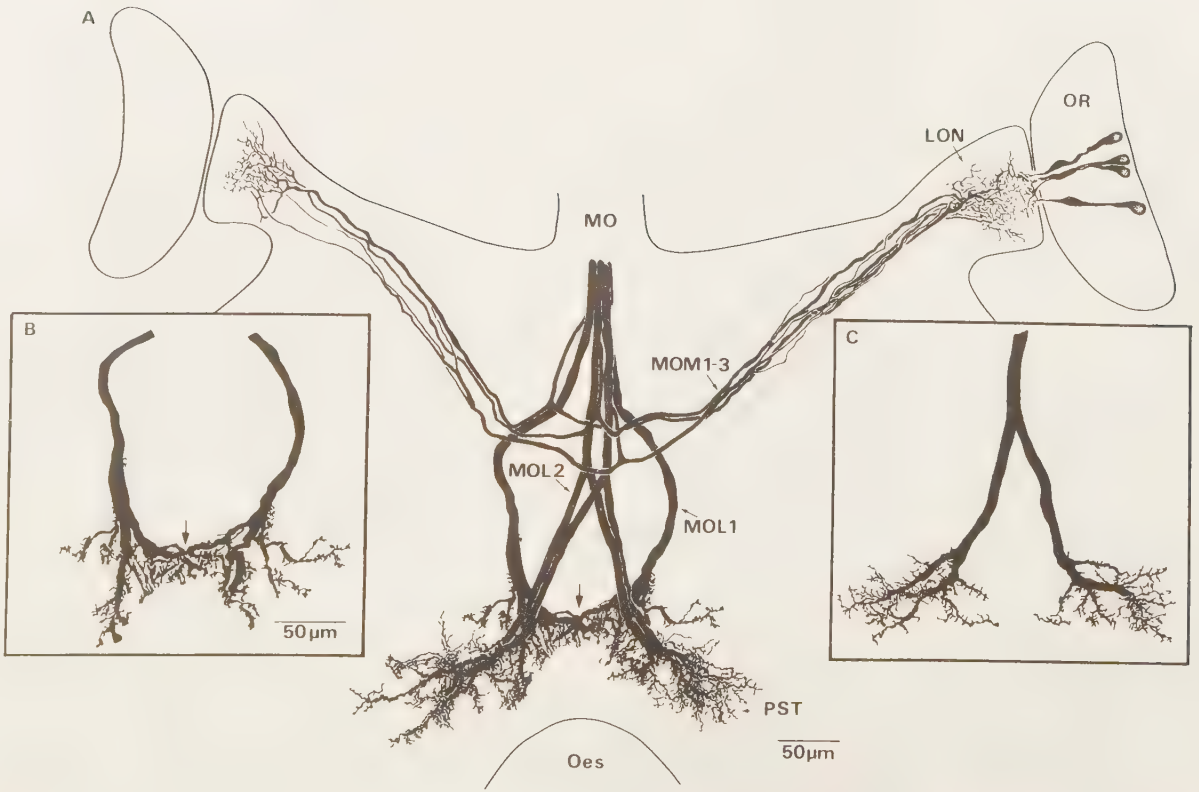

Fig 5

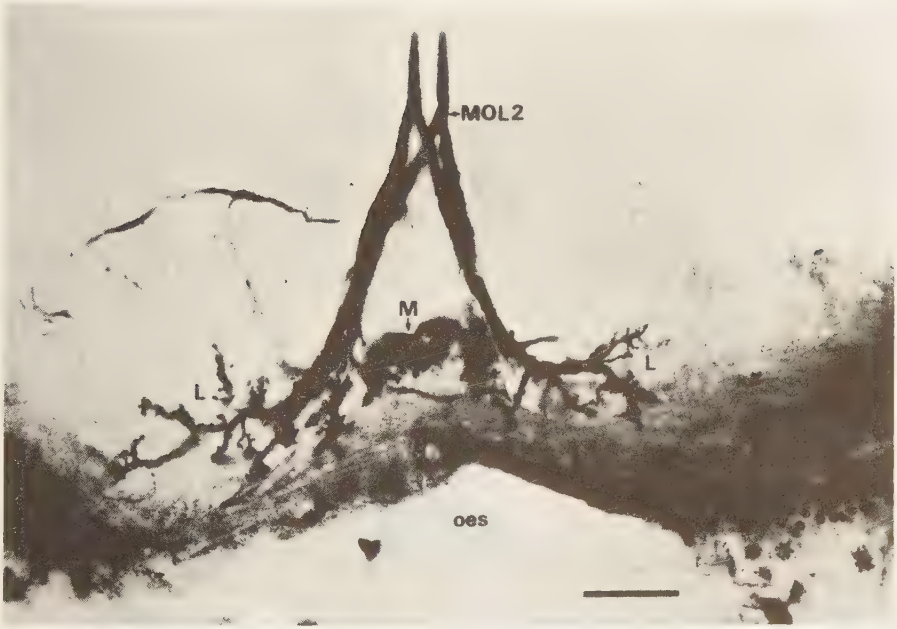

Fig 6

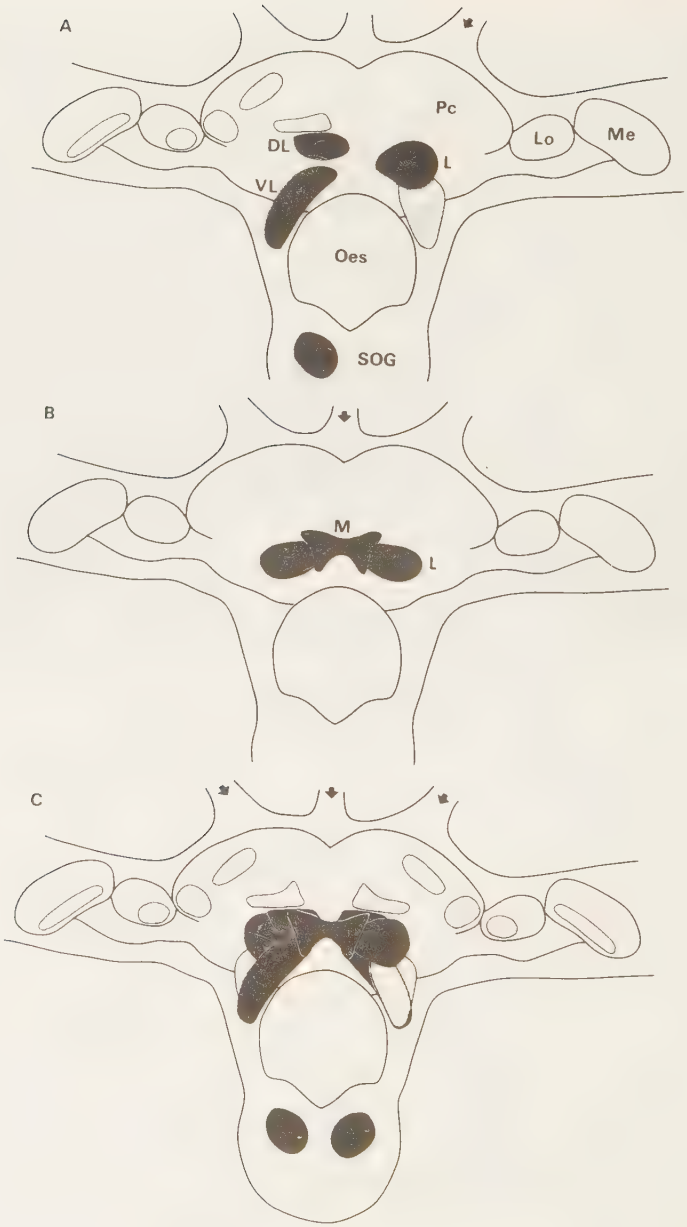

Fig 7

IV

ULTRASTRUCTURE AND CENTRAL PROJECTIONS OF EXTRAOCULAR PHOTORECEPTORS IN CADDISFLIES (INSECTA: TRICHOPTERA).

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SUMMARY

Retained larval eyes (stemmata) were studied in the imago of three representatives of Trichoptera species: Phrygania grandis, Agrypnia varia and Trichostegia minor. Light microscopically the three species appeared to represent different stages of reduction with respect to size, shape and number of lenses. However, electron-microscopic studies showed units with monolayered rhabdoms, each formed by four retinula cells in all three species. S-antigen in the retinula cells and their axons was demonstrated by use of immunocytochemistry. This method also revealed the central projections of the axons of the retinula cells, which were found to terminate either in the lamina accessoria or penetrate this area to join the fibers of the outer chiasma of the optic lobes and terminate in the medulla accessoria. The lamina accessoria and the medulla accessoria are the assumed remnants of the larval optic lobes. It is suggested that the imaginal stemmata might be still functioning photoreceptors.

Key-words: Photoreceptors, extraocular - Optic lobes - Stemmata - Immunocytochemistry - Insecta (Trichoptera).

INTRODUCTION

The larval eyes, stemmata, are retained in the imagines of many holometabolous insect species. It has been shown by ontogenetic studies that the imaginal stemmata do have their origin in the larval eyes of some species (Ephestia kühniella (Umbach 1934), Gyrinus natator substriatus (Bott 1928) and Epilachna varivestris, Adalia bipunctata (Schultz et al 1984). It is assumed that also in Trichoptera the imaginal stemmata have their origin in the larval eyes. The imaginal stemmata are reduced to differing degrees within different orders and species and their position is also variable. In representatives of Lepidoptera, they appear as a varying number of pigmented cells, which are situated inside the optic lobes between the lamina and the medulla or between the medulla and the lobula in the form of more or less compact groups of cells (Ehnbom 1948). In representatives of Coleoptera reduced larval stemmata in the imago are situated in the ventral portions of the optic lobes, between the lamina and the medulla. In these insects the rhabdoms remain well organized (Wachmann 1981). In imagines of Trichoptera larval stemmata are found in different stages of reduction (Ehnbom 1948). The reduced eyes are situated at the posterior border of the compound eyes, separated from the latter by a chitinous layer and always lie outside the optic lobes of the brain. The function of these remaining larval structures is enigmatic, but it may be assumed, based on morphological criteria, that they may represent functioning photoreceptors. It has further been proposed that imaginal stemmata are involved in the entrainment of circadian rhythms (Schultz et al. 1984).

In addition to the compound eyes the studied caddiesflies possess a second visual system, the ocelli. The morphology and ultrastructure of the ocelli will be the subject of a forthcoming report (Hallberg and Hagberg, in preparation), while the retinal and first order interneuronal projections of the ocelli of Agrypnia varia have been described in a previous report (Hagberg and Nässel 1986). The present paper is a study of the morphology and retinal projection of still another presumed photoreceptor system that exists in the head of caddiesflies. An indication that the cells of these stemmata might be functioning photoreceptors is the fact that they react with antibodies to an S-antigen. These antibodies react with photoreceptor organelles of most studied vertebrates and invertebrates (Korf et al 1985; van Veen et al 1986).

MATERIALS AND METHODS

Trichoptera of the three species Phryganía grandis Linneus (1761) Agrypnia varia Fabricius (1793) and Trichostegia minor Curtis (1834) all belonging to the family Phryganidae, were collected at Stensoffa Ecological Station, 20 km east of Lund. Adult male and female insects were caught during twilight using a mercury lamp.

For light microscopy, whole heads were fixed in Bouins fluid (Romeis 1968) for 48h, dehydrated in an alcohol series, embedded in paraffin and sectioned at a thickness of 10 µm. The sections were stained according to Delafield Halmi or Bodian (Romeis 1968).

For electron microscopy, the preparations were fixed in 2.5%

glutaraldehyde in 0.16M sodium caccodylate buffer (pH7.3) for 4h at 4°C. The specimens were washed in the same buffer and postfixed in 1% osmium tetroxide in the same buffer for 2h at 4°C. The tissue was dehydrated in alcohol and embedded in Epon. Ultrathin sections were cut with a diamond knife. The contrast of sections was enhanced by staining with uranyl acetate and lead citrate in a LKB 2168 Ultrostainer. The sections were examined in a Jeol 100CX or Zeiss 100 electron microscope.

To trace the retinal projections of the stemmata, immunohistochemistry was performed. Tissue was fixed in 4% paraformaldehyde in 0.1M phosphate buffer. After washes in buffer and infiltration with 20% sucrose, the tissue was frozen and 10 µm thick sections were cut on a cryotome. A rabbit antiserum, raised against bovine S-antigen, was used, followed by the indirect peroxidase antiperoxidase technique (PAP) for detecting S-antigen-like immunoreactivity (Sternberger 1979). The procedure and immunocytochemical controls were performed according to (Korf et al. 1985).

RESULTS

The imaginal stemmata in all three species are situated posterior to the compound eyes, beneath and detached from the cuticle of the head capsule. A small area of the cuticle situated over the stemma differs from normal cuticle in lacking microtrichs but possessing facet-like bulges (Fig.1). The stemma is situated between the lamina ganglionaris and the compound eye, separated from the latter by a cuticular fold from the surface cuticle of the head. The entire structure of the stemma is surrounded by the neurilemma of the central nervous system. A tract

connects the stemma to the optic lobes of the brain, referred to as the stemma tract. A small area of neuropil is seen next to the lamina ganglionaris, the lamina accessoria (Fig.2A). A medulla accessoria exists as well, situated next to the medulla (Fig.2C). At the light-microscopic level the three species exhibit different stages of reduction of the stemmata (Fig.2). This is, above all, manifested in the development of crystalline bodies. In P.grandis six to seven crystalline bodies are always present, whereas in A.varia and T. minor either one to four crystalline bodies may be formed or none at all. The relative size and shape of the stemmata also differ; P. grandis has a large, spheroid imaginal stemma, while in A.varia and T.minor the structure is smaller and has lost its spheroid shape attaining a wedgeshape in T. minor.

Fine structure of stemmata

The stemma of Trichostegia minor consists of a number of retinula cells roughly (40-50) surrounded and separated by glial elements, the outermost border being the neurilemma. Rounded electron-dense pigment granules (diameter: 0.7 μm) fill the retinula cells (Fig.3A). The rounded nucleus of the cell is situated distally in the cell. The cytoplasm of the cells also contains granular endoplasmic reticulum and lysosome-like bodies of variable structure. The cytoplasm contains large numbers of mitochondria close to the microvilli (Fig.3D). In the medial part of the stemma the retinula cells form microvilli, making up rhabdoms. The rhabdoms are formed by four opposed retinula cells (Fig.3B) connected by desmosomes along the edges of the rhabdoms (Fig.3E). The direction of the rhabdomeric microvilli is essentially orthogonal, each retinula cell contributing equally to the rhabdom (Fig.3E). Areas lacking regular patterns of microvilli do exist. The

crystalline body, if present, is situated close to the rhabdoms and is formed inside a special cell (Fig.3A). Cross sections of the stemma tract show axons, containing pigment granules, surrounded by glial elements (Fig.3C). The above description of T.minor agrees well with the situation in A.varia and P.grandis. Hence, the three species of Phryganidae do not show any differences in the stage of reduction at the electron-microscopic level.

Central projections of photoreceptors

The retinal axons in the two species P. grandis and A. varia were traced by immunocytochemistry using an antiserum against S-antigen. The retinal axons, surrounded by glial elements, run via the stemma tract into the optic lobe (Fig.4A). The principal portion (about 75%) of the immunoreactive axons terminates in the lamina accessoria (Fig.4B), the remainder of the axons join the fibers of the outer chiasma to terminate in the medulla accessoria (Fig.4D). In the medulla accesoria the receptor axon terminals appear as spiny bulbs (Fig.4C). Also the cell bodies and rhabdoms of the stemma cells react with the antiserum against S-antigen. Also the photoreceptor axons of the compound eyes and the ocelli react with the antisera as can be seen in Figs.4B,D.

DISCUSSION

The stemmata of the larval Trichoptera have been described by Pankrath (1890), Hesse (1901), Handlirsch and Beier (1933-36) and Paulus and Schmidt (1978). They found that the seemingly single eye on each side of the head was formed by six individual eye cups underlying the

transparent cornea. Each eye cup has its own crystalline body and bilaminar receptor layer; the distal part of the receptor layer consists of three cells and the proximal part of four cells. Basally bundles of axons project into the cerebral ganglion.

The organization of the imaginal stemmata as described here is less regular than that in the larval ones. Apparently, the larval stemmata are reduced during metamorphosis. Almost spherical crystalline bodies are formed but the total number of six is never seen. Moreover each crystalline body is formed by one cell only and, hence it is not a fused structure as in larvae. Concerning the rhabdoms no signs of a bilaminar arrangement have been seen in adult animals where four cells make up a monolayered rhabdom. The stemmata complex is however no longer juxtaposed to the cuticle. The axons of imaginal receptors do not show any signs of degeneration and the presence of S-antigen immunoreactivity indicates the presece of part of the enzymatic apparatus of rhodopsin based mechanisms which function in photoreception. The axons project to their own neuropils, the accessory lamina and the accessory medulla. These two neuropils are assumed to be the residues of the two larval visual neuropils in the imago (Ehnbom 1948). This pattern of projection where retinula axons end either in the lamina accessoria or the medulla accessoria probably reflects the patterns of projection in the larval eye. A separation of visual information by retinula axons ending at two levels (lamina and medulla) of the optic lobes is seen in the compound eyes of imagos of Trichoptera (some of these are seen in Fig.4B) and other insects (Strausfeld and Nässel 1980). In the compound eye the receptors ending in the two different layers are of different functional classes (Strausfeld and Nässel 1980).

In spite of a high density of screening pigment granules and the

detachment of the stemma from the body surface there are several reasons to believe that the stemmata are functioning light receptive organs: 1) the imaginal stemmata are situated superficially enough to be exposed to light, 2) the cuticle above the stemma is modified and appears more transparent 3) lenslike crystalline bodies lie in the light-path 4) they contain rhabdoms reacting with antibodies to S-antigen and 5) the receptor cells possess axons that are not degenerate but project to their own neuropils. It should be remarked here that the presence of immunoreactivity to S-antigen in the retained eyes conforms with the findings of van Veen et al. (1986) in other invertebrates. The intensity of the marking sometimes varies between the receptor axons of stemmata and compound eyes and ocelli in one and the same animal. The reason for this is not yet fully understood.

The suggestion (Schultz et al. 1984) that the imaginal stemmata in Epilachna varivestris (Coleoptera) might play a role in the entrainment of circadian clocks is based on the fact that in this species the structure is situated within a region of the brain where the circadian clock is thought to be located. In the ground beetle, Anthia sexguttata, this region is defined to be in or close to the lobula (Fleissner 1982, Fleissner and Fleissner 1982). A similar function of the imaginal stemmata in Trichoptera might be possible, in spite of their different location compared to E. varivestris, since the accessory medulla, where a portion of the axons of the stemmata terminate, is located close to the medulla and relatively close to the lobula. However, the precise function of this photoreceptor system must be clarified with the use of other methods. The role of other photoreceptor systems (compound eyes, dorsal ocelli) in the entrainment of circadian rhythms also remains to be solved. At least for the dorsal ocelli a similar function has been proposed (Brousse-Gaury 1971a), although

neuroanatomical studies have shown no connections between ocelli and neuroendocrine cells (Koontz and Edwards 1980).

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FIGURELEGENDS

Fig 1. Scanning electron micrograph of Agrypnia varia.

A. Cuticule (arrow) overlaying the stemma. (X 60) AB=antennal base; Co=compound eye; ocl=ocellus.

B. Magnification of area at arrow. (X 300)

Fig 2. Horizontal sections of the imaginal stemmata of A. Phrygania grandis B. Agrypnia varia C. Trichostegia minor. cr=cuticular ridge; cr b=crystalline body; la=lamina ganglionaris; me=medulla; n=neurilemma; oc=outer chiasma of optic lobes; st=stemma; Arrow in A: lamina accessoria and in C:medulla accessoria. (X 200)

Fig 3. Micrographs of the imaginal stemma of Trichostegia minor.

A. The crystalline body(cr b) its forming cell(z), the rhabdoms(rh) and the stemma tract(tr) as seen in cross section. The retinula cells are heavily pigmented. (n=neurilemma). (X 4700)

B. Rhabdom formed by four retinula cells as seen in cross section. (X 4700)

C. Cross section of the stemma tract showing pigment-granules containing

axons surrounded by glial elements. (X 22 200)

D. Enlargement of the microvilli of the rhabdoms. Note the numerous mitochondria and granular endoplasmatic reticulum close to the microvilli. (X 22 200)

E. Orthogonally arranged microvilli. At arrow desmosome connecting opposing retinula cells. (X 15 500)

Fig 4A. Schematic drawing of the imaginal stemma and its connections to the optic lobes. Axons of the retinula cells are shown terminating in the lamina accessoria(la ac) or projecting along the outer chiasma(oc) to terminate in the medulla accessoria(me ac). cr b=crystalline body (not aversed from light); la=lamina ganglionaris; lo=lobula; lop=lobula plate; me=medulla; Rh=rhabdoms of the compound eye.

B. Immunocytochemically (anti-S-antigen) marked retinula axons. Arrows mark one fiber running along the outer chiasma. (Also note the more weakly marked retinula axons of the compound eye in the lamina and the medulla. Se also Fig. 4D.) Legends as in A. (X 300)

C. Terminals of retinula axons at the medulla accessoria. (X 790)

D. Axons from the retinula cells terminating in the lamina accessoria. Legends as in A. (X 300)

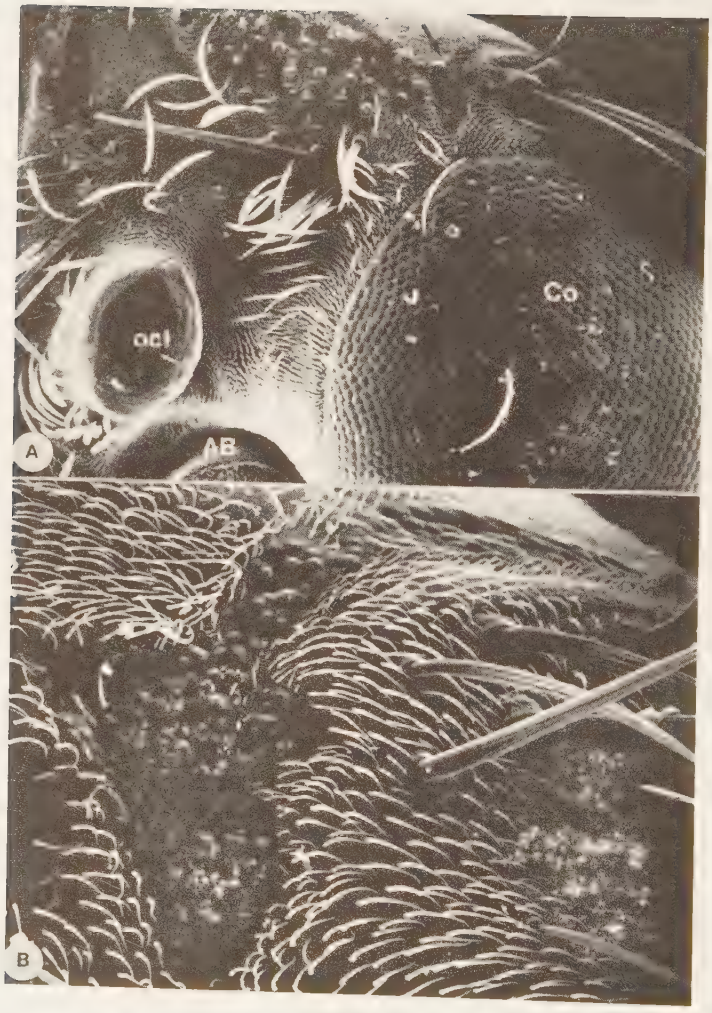

Fig 1

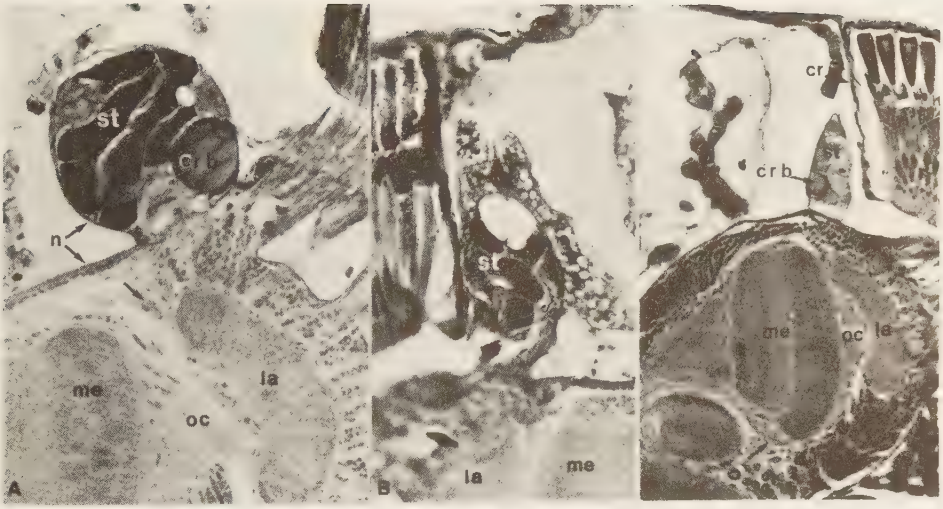

Fig 2

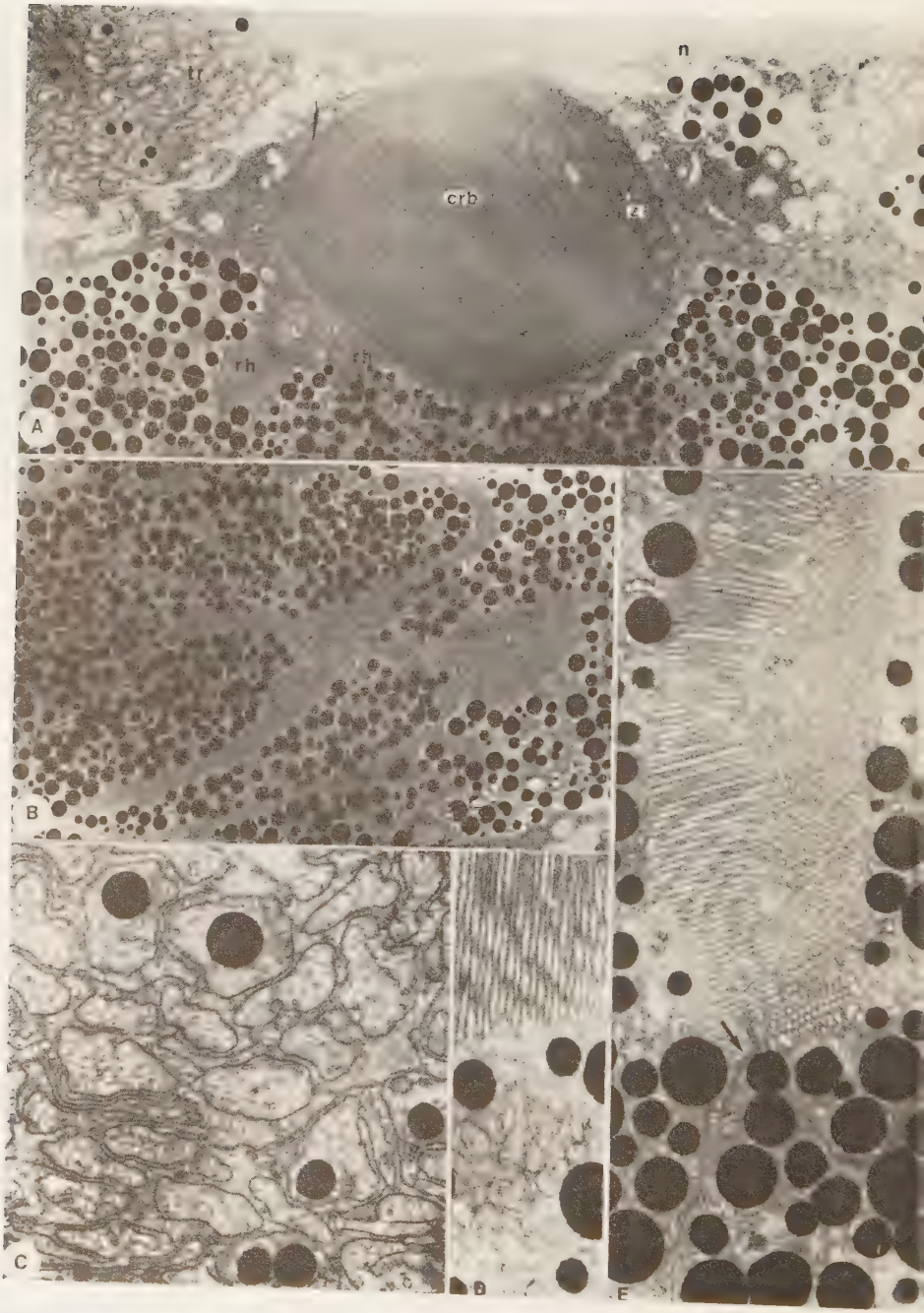

Fig 3

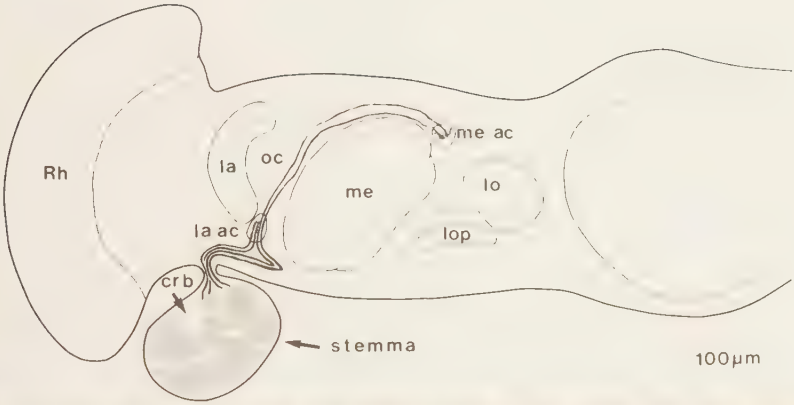
Rh
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Fig 4

Tryckericentralen
AVC i Lund 1986